REMARKS

Claims 1, 2, 6 and 8 are pending after entry of this paper. Claims 1, 2, 6 and 8 have been rejected. Claims 3-5, 7 and 9 have been cancelled without prejudice. Applicants reserve the right to pursue cancelled claims in a continuing application.

Claim 1 has been amended to incorporate the subject matter of cancelled claim 3. Support may be found throughout the instant specification, for example, claim 3 as originally filed.

Claim 2 has been amended to replace the phrase "a mouse model of Guillain-Barré syndrome" with the phrase "the transgenic mouse model." Support for this amendment may be found throughout the instant specification, for example, pages 12-13 under the "Reference Example" section.

Claim 6 has been amended to incorporate the subject matter of claim 7. Claim 6 has been further amended to recite step (iii) "observing said mouse model of the syndrome for the degree of peripheral neuropathy wherein paralysis of the tail and hind legs occurs" and the phrase "wherein said test substance has a therapeutic effect against Guillain-Barré syndrome and/or Fisher syndrome when the level of anti-GQ1b antibody and the degree of peripheral neuropathy are decreased." Support for these amendments may be found throughout the instant specification, for example, in the paragraph bridging pages 10-11.

Claim 8 has been amended to incorporate the screening method of claim 6 from which it depends.

No new matter has been introduced by these amendments. Reconsideration and withdrawal of the pending rejections in view of the above claim amendments and below remarks are respectfully requested.

Response to Rejections under 35 U.S.C. §112, first paragraph

Claims 1-3 and 6-9 stand rejected under 35 U.S.C. §112, first paragraph for lack of enablement. Specifically, the Examiner contends that the specification while being enabling for a homozygous transgenic mouse with a disruption of exon encoding S2 and EC1 of the FcγRIIB gene that does not produce FcγRIIB protein obtained after immunization with gangliosides GQ1b showing symptoms of peripheral neuropathy leading to paralysis of its tail and hind legs and elevated levels of antibody titer against GQ1b, and the method for screening test agents using such model, allegedly does not provide enablement for a model of Guillain-Barré syndrome (GBS) or Fisher syndrome or deficiency of the FcγRIIB gene or any other "mouse" showing phenotype as claimed. (Office Action – page 3). Applicants respectfully disagree.

On the contrary, the mouse model as recited in the currently amended claims would have been clearly recognized as a mouse model of Guillain-Barré syndrome by one skilled in the art in view of the technical knowledge at the time of filing and the disclosure of the instant application. Applicants clearly identify and claim three requisites that are attributed to the claimed mouse model: (a) a homozygous FcγRIIB gene deficiency; (b) elevated levels of antibody titer against GQ1b by immunizing with GQ1b ganglioside; and (c) development of peripheral neuropathy wherein paralysis of the tail and hind legs occurs. In fact, the elevated levels of antibody titer against GQ1b and peripheral neuropathy with paralysis of the tail and hind legs are known characteristic symptoms of Guillain-Barré syndrome (see paragraph 2, page 1 of the instant specification) and have not been shown to be present in any other syndrome except for Guillain-Barré syndrome or its variants. Diagnostic criteria were defined for research purposes back in 1981 (Asbury, et al., *Ann Neurol*; 3:565–6, 1978) and have been subsequently

refined in 1990 (Asbury, et al., *Ann Neurol*; 27:S21–4, 1990) as cited in Winer (*QJM: An International Journal of Medicine*, 95(11):717-721, 2002; respectfully submitted). Essentially, diagnosis requires progressive weakness of more than one limb, over a period of time, thought to be due to a neuropathy, in the absence of any identifiable genetic, metabolic or toxic cause. Whereas, elevated levels of antibodies to the ganglioside GQ1b which appear to act in part on the neuromuscular junction to interfere with transmitter release, further confirm the diagnosis of Guillain-Barré syndrome (Chiba, et al, *Neurology*; 43:1911–17, 1993; respectfully submitted). Therefore, one skilled in art would only attribute these symptoms, *i.e.*, the elevated levels of antibody titer against GQ1b and peripheral neuropathy with paralysis of the tail and hind legs, to Guillain-Barré syndrome without undue experimentation.

The fact that the Examiner contends that "hind limb or tail paralysis cannot be solely rely as phenotype for GBS or any specific syndrome" (Office Action – page 8) or "elevated level of GQ1b antibody does not provide adequate guidance in developing GBS or FS" (Office Action – page 9) fails to present any evidence to justify why both symptoms analyzed together would not be attributed to GBS or FS as disclosed in the currently amended claim 1 (incorporates the subject matter of claim 3). Examiner's contention is merely speculative and not necessarily based on any particular knowledge in the art. In fact, Reinhardt, et al. (*Adv. Exp. Med. Biol.* 1996; 398:241-6, of record), Kennel, et al. (*Neurobiol. Dis.* 1996; 3(2):137-47, art of record) Chaudhry, et al. (*Semin. Ophthalmol.* 2006; 21(4):223-27; of record), Odaka, et al. (*J. Neurol. Neurosurg. Psychiatry*, 70(1):50-55, 2001; of record) cited by the Examiner, do not refute that the symptoms such as the elevated levels of antibody titer against GQ1b and peripheral neuropathy with paralysis of the tail and hind legs taken together are characteristic symptoms of Guillain-Barré syndrome. Indeed, Chaudhry states that "[i]n the last 15 years,

attention has been directed towards the association of the GQ1b IgG antibody and several GBS variants, particularly the Fisher syndrome." (*See Id.*, Introduction, p. 223). While Odaka, et al., states that "[t]hese findings [clinical features of anti-GQ1B-IgG antibody syndrome and clinical features of each diagnostic condition] together with the common autoantibody (anti-GQ1b IgG) suggest that a common autoimmune mechanism functions in the pathogenesis of these illnesses [MFS, GBS, BBE, and acute ophthalmoparesis]" (See Odaka, et al., Abstract). The Examiner has not presented any evidence why one skilled in the art while seeing the elevated levels of antibody titer against GQ1b and peripheral neuropathy with paralysis of the tail and hind legs would not attribute these symptoms to Guillain-Barré syndrome. Thus applicants assert that one skilled in art could make and use the claimed mouse model as a model of Guillain-Barré syndrome without undue experimentation.

Furthermore, the Examiner argues that at the time of the claimed invention, neither the art of record nor the specification teaches how to practice the claimed invention for heterozygous FcγRIIB$^{+/-}$ mouse. (Office Action- page 6). Applicants respectfully wish to remind the Examiner that claim 1 recites "immunizing a homozygous FcγRIIB gene deficient mouse with GQ1b ganglioside" (emphasis added) Applicants have disclosed in the instant specification that any FcγRIIB-deficient mouse will be sufficient to practice the claimed invention. The specification must be enabling for one skilled in the art to make and use that which is defined by the claim(s) of the subject application (MPEP § 2164, [R-2], ¶ 1). Thus, the instant specification must only be enabled with respect to the homozygous FcγRIIB gene deficient mouse immunized with GQ1b ganglioside as defined by claims of the present application, which as acknowledged by the Examiner is enabling (Office Action – page 7, lines 9-12).

Furthermore, the Examiner contends that the specification allegedly does not teach any other genetic disruption involving FcγRIIB gene. Accordingly, the Examiner concludes that since the specification enables only one particular disruption of FcγRIIB$^{-/-}$ mice, *i.e.*, disruption of exon encoding S2 and EC1 of the FcγRIIB gene, any other genetic disruption will not result in the same phenotype (Office Action – page 6). Applicants respectfully disagree.

Applicants wish to bring to the Examiner's attention that "[a]s long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. 112 is satisfied." *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). "Failure to disclose other methods by which the claimed invention may be made does not render a claim invalid under 35 U.S.C. 112." *Spectral-Physics, Inc. v. Coherent, Inc.*, 827 F.2d 1524, 1533, 3 USPQ2d 1737, 1743 (Fed. Cir. 1987). The Examiner fails to cite any references or offer any evidence to justify the conclusion that any other genetic disruption of FcγRIIB$^{-/-}$ gene, will not result in the same phenotype. In fact, the art recognizes many genetic recombinant techniques that can be used to prepare any other genetic disruption to FcγRIIB gene as long as it generates a mouse whose FcγRIIB gene is deficient in its chromosome. Furthermore, MPEP §2164.01 states that a specification only needs to provide "sufficient information regarding the subject matter of the claims as to enable one skilled in the pertinent art to make and use the claimed invention." Therefore, applicants do not need to demonstrate every gene knockout method and/or gene disruption of FcγRIIB$^{-/-}$ gene to be enabling. The technology to remove the FcγRIIB gene in transgenic or knock-out non-human animals is well-known in the art. Therefore, one of ordinary skill in the art would be able to make and use the invention for any transgenic mouse with homozygous FcγRIIB-deficiency based on the description in the instant

specification and what is commonly known in the art. In addition, an artisan would understand that a transgenic mouse with homozygous FcγRIIB-deficiency does not produce FcγRIIB protein without a need for further clarification.

The Examiner further alleges while citing Holschneider et al. (*Int. J. Devl. Neuroscience* 2000; 18: 615-618) and Griffiths (*Microscopy Research and Technique* 1998; 41:344-358) that the art is unpredictable in correlating gene deficiency with a particular phenotypes (Office Action – paragraph bridging pages 6 and 7). Applicants respectfully disagree.

Applicants disclose a transgenic mouse model with a homozygous FcγRIIB gene deficiency that upon immunization with GQ1b ganglioside shows elevated levels of antibody titer against GQ1b and develops peripheral neuropathy where paralysis of the tail and hind legs occurs. Clearly, cause and effect has been established. One skilled in the art would recognize without undue experimentation that the elevated levels of antibody titer against GQ1b and the resultant phenotype, *i.e.*, a peripheral neuropathy where paralysis of the tail and hind legs occurs, were established as a result of a homozygous FcγRIIB gene deficiency and subsequent immunization with GQ1b ganglioside (*See* Examples 1 and 2 of the instant application).

Furthermore, the art teaches, for example Anagnostopoulos, et al. (*Physiol. Behav.* 2001; 73(5):675-689; respectfully submitted) that extensive knowledge of mutant phenotypes are known and identified. Anagnostopoulos, et al. describes the Transgenic and Targeted Mutation Database (TTMD) that provides an extensive, detailed phenotypic characterization of mouse mutants. (*See* Anagnostopoulos et al., at 678, first column, second paragraph). The reference further discusses the cataloging of knockout mice to serve as models for certain human disorders. (*See Id.* at 678, second column). Anagnostopoulos, et al. provides

the neurological and behavioral mouse models listed in available databases in Table 4. (See *Id.*; Table 4). Table 4 provides evidence that those skilled in the art can predict the resultant phenotypes of transgenic mice despite the assessment of Holschneider et al. and Griffiths. According to the TTMD, one can create a transgenic mouse of the type listed therein and expect to observe the recorded phenotypes.

Therefore, a specific phenotype for a mouse model with homozygous FcγRIIB gene deficiency that upon immunization with GQ1b ganglioside shows elevated levels of antibody titer against GQ1b can quite easily be confirmed by obvious visual cues (*See* Figure 1 of the instant application) such as a paralysis of the tail and hind legs and would be readily understood by one skilled in the art without undue experimentation.

With regard to the method for screening therapeutic agents for Guillain-Barré syndrome, the Examiner contends that the instant application does not provide guidance of how to identify agents that treat GBS or Fisher syndrome (Office Action – page 9). Specifically, the Examiner states that allegedly "[a]n artisan would have to perform undue experimentation to first establish a link between the immunizing transgenic animal of the invention with GQ1b with a specific syndrome and then test various parameters using different type of agents, dosage and delivery route in order to reduce symptoms seen [in] the transgenic animal of the invention" (Id. – page 9). Applicants respectfully disagree.

Applicants submit as discussed *supra*, the link between the claimed mouse model with a homozygous FcγRIIB gene deficiency having elevated levels of antibody titer against GQ1b and peripheral neuropathy with paralysis of the tail and hind legs and Guillain-Barré syndrome has been clearly established (*See* pages 5-7 of the instant response). Therefore, a substance or agent that reduces these symptoms (reduction in paralysis based on paralytic

symptom scores, *See* Example 2 and Figure 2 of the instant application) is expected to be a therapeutic drug for Guillain-Barré syndrome.

Whereas, to "test various parameters using different type of agents, dosage and delivery route in order to reduce symptoms seen [in] the transgenic animal of the invention" (*Id.* – page 9), applicants submit would be considered routine in the art and would not constitute undue experimentation since as the Examiner is well aware, undue experimentation has little to do with the quantity of experimentation and much more to do with the amount of guidance or direction provided. See *Ex parte Jackson*, 217 USPQ 804, 807 (1982) (emphasis added):

> [T]he test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed. *In re Rainer*, 52 CCPA 1593, 347 F.2d 574, 146 USPQ 218 (1965).

The specification provides sufficient guidance to test various parameters using different type of agents, dosage and delivery route in order to reduce symptoms seen in the transgenic animal of the invention. Specifically, the specification of the present application teaches methods to confirm and select therapeutic agents for Guillain-Barré syndrome by screening these therapeutic agents using the model described above. (*See Id.*, paragraph bridging pages 10 and 11). Disclosed is the administration of the test substance sought as the therapeutic agent to the mouse model either orally or parenterally. (*See Id.*). Once administered, the screening method further requires measuring the level of anti-GQ1b antibody in the blood of the non-human animal model using techniques such as Enzyme-linked Immunabsorbant assay (ELISA) analysis. (*See* Id., pages 14-15 *citing Cell. Immunol.* 145, 299-310, 1992). The

screening and selection of a therapeutic agent involves observing the mouse model for GBS-symptoms or the lack thereof. (*See Id.*, page 16).

Therefore, in light of the above arguments and amendments to the claims, applicants assert that the pending claims are enabled for a mouse model of Guillain-Barré syndrome and a method to therapeutic agents using such model. Reconsideration and withdrawal of the enablement rejection under 35 U.S.C. §112, first paragraph are respectfully requested

Response to Rejections under 35 U.S.C. §112, second paragraph

Claim 2 has been rejected under 35 U.S.C. §112, second paragraph for being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner contends that the phrase "according to" is unclear and does not further limit the instant claim (Office Action – pages 12-13). Applicants respectfully disagree.

However, in order to expedite prosecution and solely for the purpose of allowance of the instant application, applicants have amended claim 2 to clarify the antecedent basis of the mouse model recited in the claim as suggested by the Examiner. Accordingly, reconsideration and withdrawal of the 35 U.S.C. §112, second paragraph rejection of claim 2 are respectfully requested.

Claims 6-9 have been rejected under 35 U.S.C. §112, second paragraph as being incomplete for omitting essential steps. Specifically, the Examiner contends that the instant claims use a transgenic mouse for screening therapeutic agents, but allegedly does not set forth

any steps involved in the method/process (Office Action – page 13). Applicants respectfully disagree.

However, in order to expedite prosecution and solely for the purpose of allowance of the instant application, applicants have cancelled claims 7 and 9 and have amended claims 6 and 8 to recite positive steps as suggested by the Examiner. Specifically, claim 6 has been amended to incorporate the subject matter of claim 7 and further amended to recite step (iii) "observing said mouse model of the syndrome for the degree of peripheral neuropathy wherein paralysis of the tail and hind legs occurs." Furthermore, the recited steps are positively related to the preamble of the claimed method by stating "wherein said test substance has a therapeutic effect against Guillain-Barré syndrome and/or Fisher syndrome when the level of anti-GQ1b antibody and the degree of peripheral neuropathy are decreased." Support for these amendments may be found throughout the instant specification, for example, in the paragraph bridging pages 10-11. Claim 8 has been amended to incorporate the subject matter of claim 6 from which it depends. Reconsideration and withdrawal of the 35 U.S.C. §112, second paragraph rejection of claims 6-9 are respectfully requested in light of the amendments to the claims, cancellation of claims 3, 7, and 9 and above arguments.

Dependent Claims

The applicants have not independently addressed all of the rejections of the dependent claims. The applicants submit that for at least similar reasons as to why independent claim 1, from which all of the dependent claims 2, 6 and 8 depend, are believed allowable as discussed *supra*, the dependent claims are also allowable. The applicants however, reserve the

right to address any individual rejections of the dependent claims and present independent bases for allowance for the dependent claims should such be necessary or appropriate.

Thus, applicants respectfully submit that the invention as recited in the claims as presented herein is allowable over the art of record, and respectfully request that the respective rejections be withdrawn.

CONCLUSION

Based on the foregoing amendments and remarks, the applicants respectfully request reconsideration and withdrawal of the pending rejections and allowance of this application. The applicants respectfully submit that the instant application is in condition for allowance. Entry of the amendment and an action passing this case to issue is therefore respectfully requested. In the event that a telephone conference would facilitate examination of this application in any way, the Examiner is invited to contact the undersigned at the number provided. Favorable action by the Examiner is earnestly solicited.

AUTHORIZATION

The Commissioner is hereby authorized to charge any additional fees which may be required for consideration of this Amendment to Deposit Account No. **13-4500**, Order No. 4439-4032.

In the event that an extension of time is required, or which may be required in addition to that requested in a petition for an extension of time, the Commissioner is requested to grant a petition for that extension of time which is required to make this response timely and is hereby authorized to charge any fee for such an extension of time or credit any overpayment for an extension of time to Deposit Account No. **13-4500**, Order No. 4439-4032.

Respectfully submitted,
MORGAN & FINNEGAN, L.L.P.

Dated: August 17, 2007

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NEUROLOGY

Serum anti-GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain-Barré syndrome: Clinical and immunohistochemical studies

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Serum anti-GQ_{1b} IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain-Barré syndrome:

Clinical and immunohistochemical studies

A. Chiba, MD; S. Kusunoki, MD; H. Obata, MD; R. Machinami, MD; and I. Kanazawa, MD

Article abstract—To determine the significance of serum anti-GQ_{1b} IgG antibody, we studied the disease spectrum associated with this antibody and GQ_{1b} epitope in the human nervous system. We examined sera from 19 patients with typical Miller Fisher syndrome (MFS), five patients with acute postinfectious ophthalmoplegia without ataxia (atypical MFS), six patients with Guillain-Barré syndrome (GBS) with ophthalmoplegia (GBS-OP[+]), and 23 patients with GBS without ophthalmoplegia (GBS-OP[−]). We also examined sera from 84 patients with other neurologic or non-neurologic disorders and from 16 normal control subjects. Eighteen of the 19 patients with typical MFS, all the patients with atypical MFS, and five of the six patients with GBS-OP(+) had increased anti-GQ_{1b} IgG activity in ELISA, but none of the patients in the other groups, including GBS-OP(−), had it. All the patients' sera that had anti-GQ_{1b} IgG antibody showed anti-GT_{1a} IgG activity. Results of absorption studies suggested that the same antibody reacted with GQ_{1b} and GT_{1a}. An anti-GQ_{1b} mouse monoclonal antibody immunostained the paranodal regions of the extramedullary portion of the human oculomotor, trochlear, and abducens nerves. Biochemical analysis showed that the human oculomotor nerve contained a larger amount of GQ_{1b} than did the ventral and dorsal roots of the spinal cord. We conclude that serum IgG antibody against GQ_{1b} is very closely associated with acute postinfectious ophthalmoplegia in MFS and GBS.

NEUROLOGY 1993;43:1911-1917

C. Miller Fisher described[1] a syndrome consisting of ophthalmoplegia, ataxia, and areflexia (Miller Fisher syndrome [MFS]). Although, on the basis of common clinical features, he regarded it as a variant of Guillain-Barré syndrome (GBS), the relation between MFS and GBS and the pathogenesis for the features unique to MFS are unknown.

Serum antiganglioside antibodies are present in GBS.[2-5] Recently, we reported[6] the presence of serum IgG antibody against ganglioside GQ_{1b} in patients with MFS in the acute phase and pointed out an immunologic feature common to MFS and GBS: the presence of antiganglioside antibody. Serum antiganglioside antibodies also are present in, and possibly involved in the pathogenesis of, motor neuron disease (MND) and multifocal motor neuropathy (MMN).[7-11]

To evaluate the significance of serum anti-GQ_{1b} IgG antibody in detail, we investigated whether this antibody is present in larger groups of patients that included those with atypical MFS and GBS with ophthalmoplegia. We also investigated the distribution of the epitope for anti-GQ_{1b} antibody immunohistochemically using a mouse monoclonal antibody (mAb).

Methods. *Examination of antiglycolipid antibody in patients' sera.* Patients. Serum samples were obtained from 19 patients with typical MFS, all of whom had the triad of the syndrome (ophthalmoplegia, ataxia, and hyporeflexia) but no major limb weakness or other signs suggestive of CNS involvement. We also examined five patients who experienced acute ophthalmoplegia after an infectious prodrome (four infected with common cold and one with diarrhea) who recovered without specific therapy. They all had a relatively mild bilateral abducens palsy; two had hyporeflexia, two a mild decrease of vibration sense in the lower extremities, and two a mild albuminocytologic dissociation in the cerebrospinal fluid, but

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Table. Clinical features of patient groups

	No. pts	Ophthalmoplegia Ext	Ophthalmoplegia Int	Ataxia	Hypo- or areflexia	Limb weakness	First serum examination* After prodrome	First serum examination* After onset
Typical MFS	19	+	− or +	+	+	− or ±	5-37	1-21
Atypical MFS	5	+	−	−	− or +	−	14-64	6-60
GBS-OP(+)	6	+	− or +	−, +, or ?	+	+	14-63	5-53
GBS-OP(−)	23	−	−	−, +, or ?	+	+	9-40	1-20

* Range in days.
Ext External ophthalmoplegia.
Int Internal ophthalmoplegia.
MFS Miller Fisher syndrome.
GBS-OP(+) Guillain-Barré syndrome (GBS) with ophthalmoplegia.
GBS-OP(−) GBS without ophthalmoplegia.
\+ Present.
− Absent.
± Minimal.
? Unable to be assessed because of limb weakness.

none had ataxia or any other neurologic sign. This group was designated "atypical MFS."

Serum samples also were taken from six patients who had GBS and ophthalmoplegia (GBS-OP[+]). Twenty-three patients with GBS but without ophthalmoplegia (GBS-OP[−]) also were examined, three of whom showed ataxia that was considered to be due to loss of position sense. In the others, ataxia was absent or could not be assessed because of limb weakness. The clinical features of these patients are given in the table. Neurologists examined them in the acute phase. The first serum samples were taken in the acute phase, except for one patient with atypical MFS and one patient with GBS-OP(+), from whom samples were obtained 60 days (atypical MFS) and 53 days (GBS-OP[+]) after neurologic onset.

As disease controls, serum samples were taken from two patients with brainstem encephalitis, 24 with MS, five with MMN, 12 with chronic inflammatory demyelinating polyneuropathy (CIDP), and 23 with MND, as well as 18 patients with other immunologic disorders, nine of whom had systemic lupus erythematosus, seven polymyositis, and two mixed connective tissue disorder. Except for MND, the samples were taken in the acute, active, or relapse phases of the disorders. Samples also were taken from 16 normal control subjects. Both patients with brainstem encephalitis had lesions in the brainstem, as confirmed by MRI, showing abnormal ocular movement (conjugate gaze palsy in one, coarse horizontal gaze nystagmus in the other), ataxia, and hyporeflexia, as well as consciousness disturbance and dysarthria. They eventually recovered. Serologic examinations suggested herpes simplex virus infection in one of them, but the causative agent in the other is not known. Of the 24 patients with MS, nine had ataxia and four ophthalmoplegia or diplopia. All five patients with MMN had the serum anti-GM_1 IgM antibody. None of the patients with CIDP had ophthalmoplegia.

Enzyme-linked immunosorbent assay (ELISA). All the glycolipids except GQ_{1b} and asialo-GM_1 (GA_1) were purchased from Funakoshi (Tokyo, Japan). GQ_{1b} was bought from Dia-Iatron (Tokyo, Japan). GA_1 was prepared from the GM_1 of bovine brain in our laboratory. The titer of the serum anti-GQ_{1b} IgG antibody was determined by ELISA. Five hundred ng of purified GQ_{1b} in 50 μl of ethanol was added to individual wells of Linbro microtiter plates (Flow Laboratories, McLean, VA), after which the solution was dried by evaporation. Nonspecific protein-binding sites were saturated with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), pH 7.4 (blocking solution). A 50-μl serum sample diluted serially from 1:10 with blocking solution was added in duplicate to the antigen-coated and -uncoated wells, after which the plates were incubated overnight at 4 °C. The wells then were washed three times with the blocking solution and incubated for 2 hours at room temperature with 50 μl of peroxidase-conjugated goat anti-human IgG antibody (γ-chain specific; Cappel, West Chester, PA) diluted 1:500 with blocking solution. Next, they were washed three times then incubated with 200 μl of 40 mg/dl of o-phenylenediamine dihydrochloride and 0.006% H_2O_2 in phosphate-citrate buffer, pH 5.0, as the enzyme substrate. The reaction was stopped by the addition of 50 μl of 8N H_2SO_4. The color reaction was read at 492 nm with an ELISA reader (BioRad, Hercules, CA). Readings for the GQ_{1b}-uncoated wells were subtracted from those for the GQ_{1b}-coated wells. The titer for each patient was taken as the highest dilution factor at which the mean OD of the duplicate wells exceeded the cutoff value. This corresponded to the mean + 3 SD of the normal control sera at the dilution of 1:10. Taking into account the variations of the OD values in the different experiments, we determined the cutoff value for each assay by using serially diluted positive standard serum on the same plate. This value ranged from 0.215 to 0.350 in the anti-GQ_{1b} IgG antibody assay. Serum anti-GQ_{1b} IgM antibody and the IgM and IgG antibodies against other glycolipids (GM_1, GM_2, GM_3, GD_{1a}, GD_{1b}, GD_3, GT_{1b}, GA_1, galactocerebroside) also were examined by ELISA, as described earlier, at a serum dilution of 1:40. For the second antibody in the IgM class, we used peroxidase-conjugated goat antihuman IgM antibody (μ-chain specific; Cappel) diluted 1:200. The cutoff value also corresponded to the mean + 3 SD of the normal control sera for each glycolipid.

Thin-layer chromatography (TLC) and enzyme immunoassay. GM_1, GD_{1a}, GD_{1b}, GT_{1b}, GQ_{1b}, and GT_{1a} were the test antigens used. GT_{1a} was the gift of Dr. Susumu Ando, Tokyo Metropolitan Institute of Gerontology. A 500-ng sample of each purified ganglioside was loaded on a plastic-backed TLC plate (Macherey-Nagel, Düren, Germany) and developed with chloroform: methanol:0.2% $CaCl_2$ (45:55:10). The plate then was air-dried, dipped in a solution of 0.4% polyisobutylmethacrylate in *n*-hexane, and air-dried again. Nonspecific protein-binding sites were saturated with 10% normal goat serum in PBS. The different lanes on the plate were overlaid with sera from the patients or normal control subjects diluted with 10% normal goat serum in PBS: 1:40 for patients with anti-GQ_{1b} IgG antibody titers above 1:160 in ELISA and 1:20 for subjects with titers

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below 1:80. The plate then was incubated overnight at 4 °C, after which it was washed with PBS and incubated for 2 hours at room temperature with peroxidase-conjugated goat antihuman IgG antibody diluted 1:200. Immunoreactants were made visible with PBS containing 50 mg/dl 3,3′-diaminobenzidine tetrahydrochloride and 0.01% H_2O_2.

Absorption study. Antiglycolipid antibodies were absorbed in the antigen-coated ELISA wells. Each well of the microtiter plate was coated with 500 ng of purified glycolipid. Nonspecific protein-binding sites were saturated as described earlier. Fifty μl of sera diluted with 10% normal goat serum in PBS, in the same ratio used for the TLC-enzyme immunoassay, was added to both the antigen-coated and -uncoated wells, after which the plates were incubated overnight at 4 °C. The residual antiglycolipid activities of the supernatants were assayed by TLC-enzyme immunoassay and the activities absorbed in the wells were assayed by ELISA. We confirmed there were no significant differences in the protein concentrations of the materials absorbed by this method.

Immunohistochemical and biochemical studies. We used tissue specimens obtained from autopsied patients with no known neurologic disorders. These specimens were frozen within 18 hours of death and kept at −80 °C until used.

Immunohistochemical study. Human nerve tissues were studied immunohistochemically with a monoclonal antibody, mAb 7F5, that reacts with both GQ_{1b} and GT_{1a} and belongs to the IgG_{2a} subclass of the mouse.[12] This antibody was provided by Mecto (Tokyo, Japan). We examined all 12 cranial nerves and the ventral and dorsal roots of the lumbar spinal cord, as well as the femoral nerve, dorsal root ganglion (DRG), thoracic spinal cord, cerebellum, and brainstem. Specimens were frozen in isopentane cooled in liquid nitrogen. Sections of 10 μm were cut on a cryostat at −25 °C, then mounted on gelatin-coated slide glasses, air dried, and fixed in cold acetone for 5 minutes, after which they were immunostained with Vectastain ABC kit (Vector Laboratories, Burlingame, CA). After incubating them with 1.5% normal horse serum, the sections were incubated overnight at 4 °C with mAb 7F5 diluted 1:400 (corresponding to 15 μg of IgG_{2a} per ml), after which they were washed twice with PBS (10 minutes each), then incubated for 2 hours at room temperature with biotinylated horse antimouse IgG antibody diluted 1:200. After two washings with PBS, the sections were incubated for 1 hour at room temperature with the avidin-biotin complex. After two more PBS washes, the immunoreactants were made visible with 50 mg/dl of 3,3′-diaminobenzidine tetrahydrochloride and 0.01% H_2O_2 in PBS. Purified mouse IgG_{2a} (Chemicon, Temecula, CA) diluted to 15 μg/ml was the control.

Ganglioside preparation. The total ganglioside fractions were prepared from the oculomotor nerve and the ventral and dorsal roots of the lumbar spinal cord. About 100 mg of each tissue was homogenized. The total lipid fractions were extracted sequentially with 3 ml each of chloroform:methanol:water at 2:1:0, 1:1:0, and 1:2:0.8 (by volume). The extract was evaporated serially on a rotary evaporator. Its residue was dissolved in 2 ml of chloroform:methanol (9:1), then applied to a Phenyl Sepharose column (Pharmacia, Uppsala, Sweden) (column volume, 2 ml). Neutral lipids and sulfatides were eluted with 10 ml each of chloroform:methanol (neutral lipids at 9:1 and sulfatides at 85:15). The total gangliosides were recovered with acidic phospholipids on elution with 10 ml of chloroform:methanol (1:1) and 20 ml of methanol.[13] The eluate obtained was evaporated in a rotary evaporator. The residue was given mild alkaline treatment with 0.5 M sodium hydroxide in methanol for 1 hour at 45 °C to degrade acidic phospholipids. After neutralization with 1N acetate in methanol, the sample was evaporated under nitrogen. The residue was dissolved in 500 μl of chloroform:methanol:water (5:5:1) and applied to a gel chromatography column of Toyopearl HW-40 (Toso, Tokyo, Japan) (column volume, 10 ml) to remove salts. The first 3 ml of the eluate was discarded. The second 3 ml was collected and evaporated under nitrogen, after which the residue was dissolved in chloroform:methanol (1:1) at a concentration of 1 mg of original tissue per 4 μl. The ganglioside patterns of samples were made visible with resorcinol-HCl reagent after chromatography on an HPTLC plate (Merck, Darmstadt, Germany) with chloroform:methanol:0.2% $CaCl_2$ (45:55:10).

TLC-enzyme immunoassay. TLC-enzyme immunoassays of the total ganglioside fractions from the oculomotor nerve and the ventral and dorsal roots of the spinal cord were done as described earlier. A sample corresponding to 2.5 mg wet weight of tissue was loaded on a plastic-backed TLC plate. After the plate had been developed, it was incubated with mAb 7F5 (1:200) or a patient's serum (1:40) for 1.5 hours at room temperature. The second antibody for mAb 7F5 was peroxidase-conjugated goat antimouse IgG antibody (1:200) (Cappel) and, for the patient's serum, peroxidase-conjugated goat antihuman IgG antibody (1:200).

Western blot analysis. Specimens were homogenized in ice-cold modified Laemmli's sample buffer containing 0.08 M Tris-HCl (pH 6.8), 2% sodium dodecyl sulfate (SDS), 10% glycerol, and 0.1 M dithiothreitol. After the samples had been immersed in boiling water for 1.5 minutes, insoluble precipitates were removed by centrifugation at 10,000 × *g* and 4 °C for 15 minutes. The protein concentrations then were measured by the method of Lowry et al,[14] as modified by Ross and Schatz,[15] with BSA as the standard. Twenty μg of protein per lane was separated by electrophoresis on a 15% SDS-polyacrylamide slab gel, then transferred electrophoretically to a nitrocellulose membrane.[16,17] The blots were immunostained by enzyme-immunoassay with ProtBlot (Promega, Madison, WI). After incubation with 3% BSA for 6 hours at room temperature, the membrane was incubated overnight at 4 °C with the first antibody diluted with Tris-buffered saline (pH 8.0) containing 0.05% Tween 20. After being washed, the membrane was incubated for 2 hours at room temperature with alkaline phosphatase-conjugated antimouse IgG diluted 1:7,500. The immunoreactants were made visible with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as the enzyme substrates. MAb 7F5 and purified mouse IgG_{2a} (the control) were used as the first antibodies at 15 μg of IgG_{2a} per ml.

Results. *Examination of antiglycolipid antibody in patients' sera.* Eighteen of the 19 patients with typical MFS, all five of those with atypical MFS, and five of the six with GBS-OP(+) had increased anti-GQ_{1b} IgG antibody titers ranging from 1:40 to 1:640 in ELISA (figure 1). In three of these patients, we could examine their sera as early as 1 or 2 days after neurologic onset, and they all had high titer of 1:320. None of the 23 patients with GBS-OP(−), the 84 disease control subjects, or the 16 normal control subjects had titers above 1:10.

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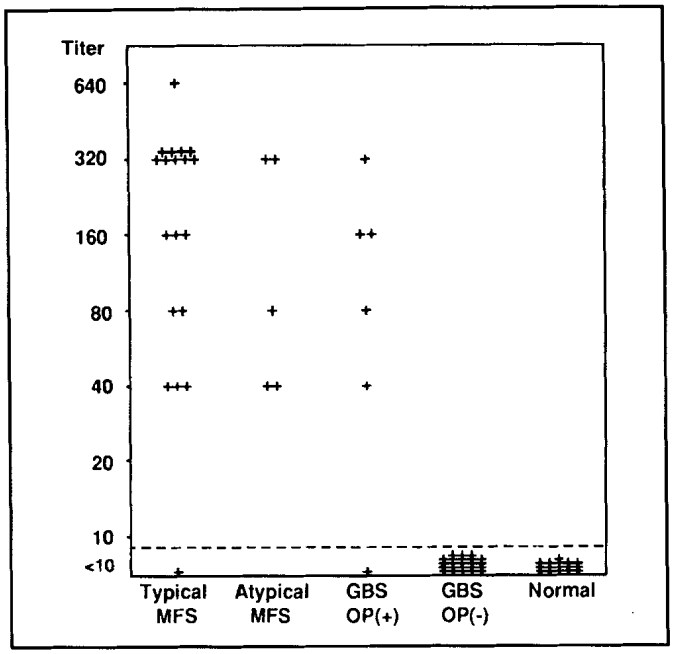

Figure 1. Anti-GQ_{1b} IgG antibody titers. Eighteen of the 19 patients with typical Miller Fisher syndrome (MFS), all five patients with atypical MFS, and five of the six patients with Guillain-Barré syndrome with ophthalmoplegia (GBS-OP[+]) show increased titers ranging from 1:40 to 1:640. The titers of patients with GBS without ophthalmoplegia (GBS-OP[−]) and those of the normal subjects are all less than 1:10.

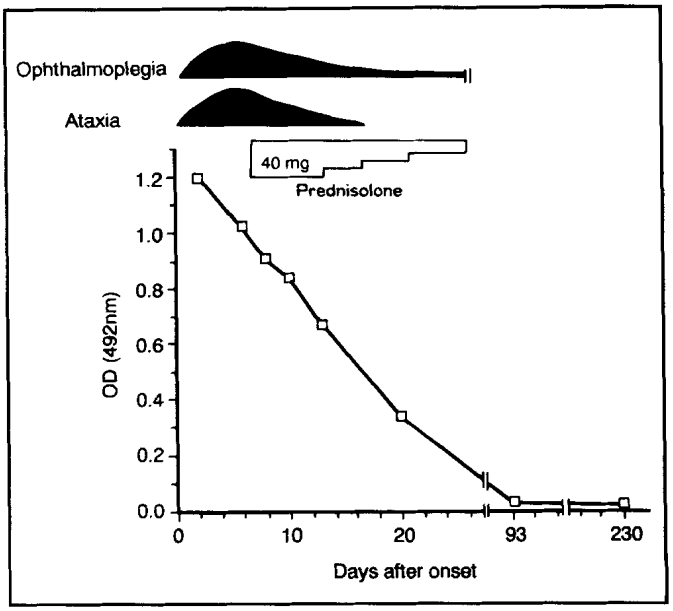

Figure 2. Profiles of the clinical manifestations and anti-GQ_{1b} IgG activity, at a serum dilution of 1:80, of a patient with typical MFS. Ophthalmoplegia represents the degree of diplopia in lateral gaze, and ataxia, unsteadiness of gait. The ophthalmoplegia was bilateral abducens palsy. Anti-GQ_{1b} IgG activity is present 2 days after neurologic onset but decreases during the progression phase before the administration of prednisolone.

In all 28 patients with anti-GQ_{1b} IgG antibody, anti-GT_{1a} IgG antibody also was detected in the TLC-enzyme immunoassay. The other antiglycolipid activities were detected in five of them: anti-GD_{1b} IgG activity in two (one with typical MFS, the other with GBS-OP[+]); anti-GD_3 IgG activity in one with GBS-OP(+); anti-GD_{1b} and relatively weak anti-GD_3 IgG activities in one with typical MFS (who also had IgM activities against GQ_{1b}, GM_1, and GD_{1b}); and anti-GQ_{1b} IgM activity in one with atypical MFS.

To investigate the cross-reactivity of the anti-GQ_{1b} and anti-GT_{1a} IgG antibodies, we did absorption studies on four patients: two with typical MFS, one with atypical MFS, and one with GBS-OP(+). Preliminary incubation with GQ_{1b} or GT_{1a} reduced the antibody activities against both GQ_{1b} and GT_{1a} in the sera of all the patients.

Serial studies of anti-GQ_{1b} IgG were done on 18 patients, five of whom were given prednisolone orally and three of whom received plasmapheresis therapy. The first serum samples had the highest anti-GQ_{1b} IgG activities, which decreased with time whether or not prednisolone or plasmapheresis treatment had been used. No patient showed a reincrease in anti-GQ_{1b} IgG activity except for one who showed a little reincrease in the activity without clinical exacerbation in association with the nonspecific rebound of the total serum IgG concentration after extensive plasmapheresis. Profiles of the anti-GQ_{1b} IgG activity and the clinical manifestations of one patient from whom sera could be obtained at short intervals from 2 days after neurologic onset are shown in figure 2. Although his clinical manifestations worsened during the first 6 days after onset, anti-GQ_{1b} IgG antibody activity was highest in the first sampling taken 2 days after onset, decreasing even in the phase when his clinical signs were exacerbated before prednisolone administration.

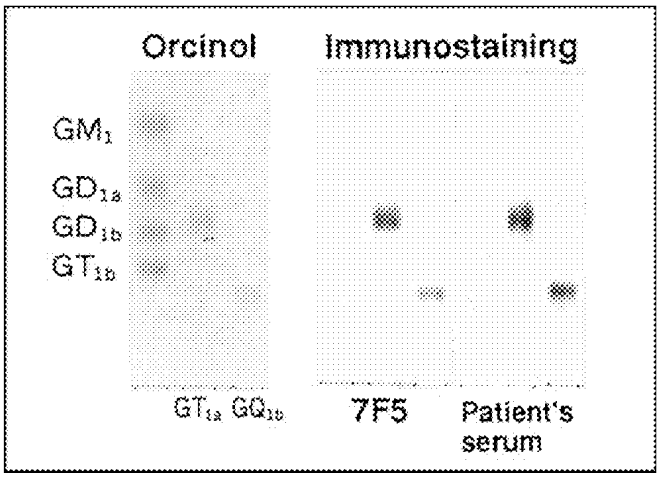

Figure 3. Binding characteristics of mAb 7F5. MAb 7F5 has the same specificity as the patient's sera. It reacts with GQ_{1b} and GT_{1a} but not with GM_1, GD_{1a}, GD_{1b}, or GT_{1b}.

Immunohistochemical and biochemical studies. Binding characteristics of mAb 7F5 are shown in

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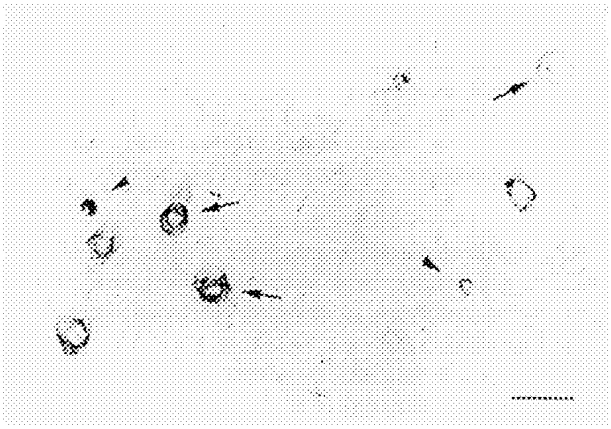
Figure 4. Oculomotor nerve immunostained with an anti-GQ_{1b} monoclonal antibody, mAb 7F5. (A) Cross section. The many doughnut-shaped stainings appear to consist of unstained axoplasm surrounded by a stained portion. The entire surrounding portion is stained in some of them (arrows) and the inner part only in others (arrowheads). (Bar = 25 μm.) (B) Longitudinal section. The paranodal regions of Schwann cells mainly appear to be stained. (Bar = 10 μm.)



A

B

figure 3. This mAb recognizes GQ_{1b} and GT_{1a} but not GM_1, GD_{1a}, GD_{1b}, or GT_{1b}. Purified mouse IgG_{2a} used as the isotype control did not react with any of the gangliosides.

Unique immunostaining by mAb 7F5 was seen in the oculomotor, trochlear, and abducens nerves in both the proximal portion of the subarachnoid space and the distal portion adjacent to the external ocular muscles. Doughnut-shaped stainings were present in cross section (figure 4A). In longitudinal sections, the paranodal regions were immunostained (figure 4B). These stainings were lessened by prior treatment of a section with chloroform:methanol (1:1) for 1 minute. Similar stainings were not, or only rarely, present in the other peripheral nerve tissues. Some large DRG cells had granular stainings in their cytoplasms. In the CNS, the gray matter in the spinal cord and brainstem and the deep cerebellar nuclei generally had faint stains, but these were much weaker than the staining of the oculomotor, trochlear, and abducens nerves. No nerve fiber bundles in the spinal cord and brainstem, including the intramedullary portions of the oculomotor and abducens nerves, were stained.

Analysis of the total ganglioside fractions showed that the human oculomotor nerve had GQ_{1b}, but no GT_{1a} detectable in this condition (figure 5). Although GQ_{1b} was present in both the ventral and dorsal roots, its content was greater in the oculomotor nerve for the same wet weight of tissue. In the TLC-enzyme immunoassay of the total ganglioside fractions, both mAb 7F5 and the patient's serum immunostained only GQ_{1b} among the gangliosides. No specific bands were detected by the Western blot analysis.

Discussion. We confirmed the previously reported serum anti-GQ_{1b} IgG antibody in patients in the acute phase of typical MFS.[6] Moreover, we found anti-GQ_{1b} IgG antibody in patients with atypical MFS and those with GBS-OP(+), whereas no patient with GBS-OP(−) had it. Although the grouping of these patients was arbitrary, the correlation with the clinical manifestations suggests that this antibody is most closely associated with acute ophthalmoplegia in MFS and GBS.

We detected anti-GT_{1a} IgG activity in all patients with anti-GQ_{1b} IgG activity. The absorption studies showed that the anti-GQ_{1b} and anti-GT_{1a} antibodies cross-reacted with each other's antigen, which suggested that the same IgG antibody bound to both antigens.

Serum anti-GQ_{1b} activities were present in patients with IgM paraproteinemia and sensory-dominant neuropathy,[18,19] but presumably without ophthalmoplegia, the anti-GQ_{1b} activities possibly being derived from IgM M proteins binding to a broad spectrum of polysialogangliosides. In our series, the IgG antibodies against GQ_{1b} and GT_{1a} in patients with typical and atypical MFS and with GBS-OP(+) reacted with none of the other gangliosides examined or, at most, only with a very few

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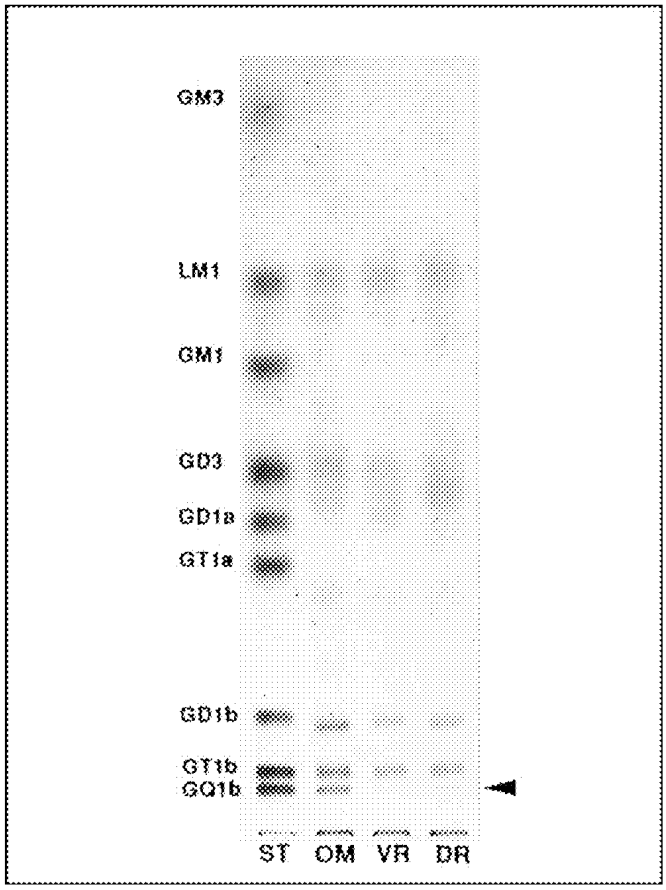

Figure 5. Ganglioside patterns. Ganglioside fractions corresponding to 4.0 mg wet weight of tissue were chromatographed on an HPTLC plate then stained with resorcinol. The oculomotor nerve (OM) has more GQ_{1b} than do the ventral root (VR) and dorsal root (DR) of the lumbar spinal cord for the same wet weight (arrowhead). ST = mixture of known standard gangliosides (500 ng each).

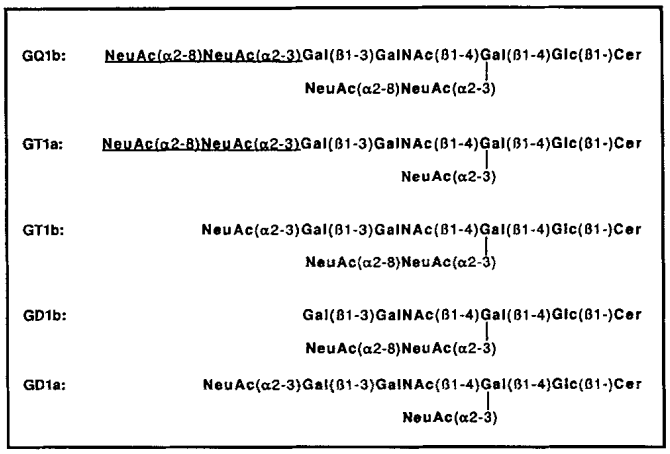

Figure 6. Structures of the gangliosides GQ_{1b}, GT_{1a}, GT_{1b}, GD_{1b}, and GD_{1a}. GQ_{1b} and GT_{1a} have a disialosyl residue in common that is attached to the nonreducing terminal of the gangliotetraose structure (underlined).

(GD_{1b}, GD_3, or GM_1) in four of the 28 patients, but never with GT_{1b} and GD_{1a}. The disialosyl residue attached to the nonreducing terminal of the gangliotetraose structure (figure 6, underlined) may be critical for the anti-GQ_{1b} antibody associated with ophthalmoplegia.

In the typical MFS, atypical MFS, and GBS-OP(+) groups, two patients (one with typical MFS, the other with GBS-OP[+]) did not have the anti-GQ_{1b} IgG antibody. Both of these anti-GQ_{1b}–negative patients had isolated complete unilateral abducens palsy, whereas the patients with anti-GQ_{1b} IgG antibody had bilateral ophthalmoplegia: 14 total external ophthalmoplegia, 13 bilateral abducens palsy with or without other impairment, and one bilateral palsy of adduction and infraduction. Although abducens palsy is the most common early extraocular sign in MFS and GBS, isolated complete unilateral abducens or oculomotor palsy, as in the two patients in our series, is rare.[20] The underlying mechanism of ophthalmoplegia in these two patients without anti-GQ_{1b} antibody may differ from that in patients with the antibody.

The immunohistochemical study with the anti-GQ_{1b} mAb showed prominent staining in the oculomotor, trochlear, and abducens nerves that was not present elsewhere in the nervous system examined. Biochemical analysis showed that the oculomotor nerve had a higher content of ganglioside GQ_{1b} than did the ventral and dorsal roots of the lumbar spinal cord for the same wet weight of tissue. Taking into account the negative results of Western blot analysis and the positive results of the TLC-enzyme immunoassay, we suspect that the mAb recognized ganglioside GQ_{1b} in the oculomotor nerve. The unique distribution of GQ_{1b} is compatible with the clinical association of anti-GQ_{1b} antibody with ophthalmoplegia.

The GQ_{1b} epitope was expressed mainly in the paranodal regions of the extramedullary portion of the three cranial nerves involved in ocular movement. The paranodal regions of Schwann cells have special features indicative of their involvement in impulse generation,[21,22] and damage to these regions blocks impulse generation at the nodes of Ranvier.[23] These findings suggest the probable attack site of the anti-GQ_{1b} antibody and the cause of the conduction block. The extramedullary distribution of the attack site is compatible with the neuropathologic findings in a patient with GBS with ophthalmoplegia who had demyelinating lesions in the extramedullary portions of the oculomotor and abducens nerves.[24]

Although anti-GQ_{1b} antibody might be elevated as a result of tissue destruction, we feel it is involved in pathogenesis because of the high titer as early as 1 or 2 days after neurologic onset. Close serial examination of one patient showed that the peak of anti-GQ_{1b} IgG activity preceded the maximum neurologic manifestations. In another patient, anti-GQ_{1b} IgG antibody reincreased transiently, without clinical exacerbation, in association with the nonspecific rebound of the total serum IgG concentration after extensive plasmapheresis, suggesting that the damage was not dependent on the intensity of the antibody activity alone. A time lag

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between the peaks of antibody activity and the symptoms would be expected if the antibody is responsible for the initiation of damage.

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References

1. Fisher CM. An unusual variant of acute idiopathic polyneuritis (syndrome of ophthalmoplegia, ataxia and areflexia). N Engl J Med 1956;255:57-65.
2. Ilyas AA, Mithen FA, Dalakas MC, Chen ZW, Cook SD. Antibodies to acidic glycolipids in Guillain-Barré syndrome and chronic inflammatory demyelinating polyneuropathy. J Neurol Sci 1992;107:111-121.
3. Svennerholm L, Fredman P. Antibody detection in Guillain-Barré syndrome. Ann Neurol 1990;27(suppl):S36-S40.
4. Yuki N, Yoshino H, Sato S, Miyatake T. Acute axonal polyneuropathy associated with anti-GM_1 antibodies following *Campylobacter* enteritis. Neurology 1990;40:1900-1902.
5. Kusunoki S, Chiba A, Shimizu T, Yamada H, Mannen T. Anti-glycolipid antibody in Guillain-Barré syndrome. In: Yonezawa T, ed. Satellite symposium on demyelination, mechanisms and background. Kyoto, Japan: XI International Congress of Neuropathology, 1990:13-19.
6. Chiba A, Kusunoki S, Shimizu T, Kanazawa I. Serum IgG antibody to ganglioside GQ_{1b} is a possible marker of Miller Fisher syndrome. Ann Neurol 1992;31:677-679.
7. Freddo L, Yu RK, Latov N, et al. Gangliosides GM_1 and GD_{1b} are antigens for IgM M-protein in a patient with motor neuron disease. Neurology 1986;36:454-458.
8. Pestronk A, Cornblath DR, Ilyas AA, et al. A treatable multifocal motor neuropathy with antibodies to GM_1 ganglioside. Ann Neurol 1988;24:73-78.
9. Schluep M, Steck AJ. Immunostaining of motor nerve terminals by IgM M protein with activity against gangliosides GM_1 and GD_{1b} from a patient with motor neuron disease. Neurology 1988;38:1890-1892.
10. Thomas FP, Thomas JE, Sadiq SA, et al. Human monoclonal IgM anti-Gal(β1-3)GalNAc autoantibodies bind to the surface of bovine spinal motoneurons. J Neuropathol Exp Neurol 1990;49:89-95.
11. Santoro M, Thomas FP, Fink ME, et al. IgM deposits at nodes of Ranvier in a patient with amyotrophic lateral sclerosis, anti-GM_1 antibodies, and multifocal motor conduction block. Ann Neurol 1990;28:373-377.
12. Yamamoto H, Tsuji S, Nagai Y. Tetrasialoganglioside GQ_{1b} reactive monoclonal antibodies: their characterization and application for quantification of GQ_{1b} in some cell lines of neuronal and adrenal origin(s). J Neurochem 1990;54:513-517.
13. Ando S, Waki H, Kon K, Tanaka Y, Kishimoto Y. Isolation of minor gangliosides and their structural analysis. In: Ledeen RW, Hogan EL, Tettamanti G, Yates AJ, Yu RK, eds. New trends in ganglioside research: neurochemical and neuroregenerative aspects. Fidia research series vol 14. Padua: Liviana Press, 1988:27-35.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-275.
15. Ross E, Schatz G. Assay of protein in the presence of high concentrations of sulfhydryl compounds. Anal Biochem 1973;54:304-306.
16. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-685.
17. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979;76:4350-4354.
18. Yuki N, Miyatani N, Sato S, et al. Acute relapsing sensory neuropathy associated with IgM antibody against B-series gangliosides containing a GalNAcβ1-4 (Gal3-2αNeuAc8-2αNeuAc)β1 configuration. Neurology 1992;42:686-689.
19. Obi T, Kusunoki S, Takatsu M, Mizoguchi K, Nishimura Y. IgM M-protein in a patient with sensory-dominant neuropathy binds preferentially to polysialogangliosides. Acta Neurol Scand 1992;86:215-218.
20. Ropper AH, Wijdicks EFM, Truax BT. Guillain-Barré syndrome. Contemporary neurology series, vol. 34. Philadelphia: FA Davis, 1991:90-92, 114-115.
21. Ellisman MH, Friedman PL, Hamilton WJ. The localization of sodium and calcium to Schwann cell paranodal loops at nodes of Ranvier and of calcium to compact myelin. J Neurocytol 1980;9:185-205.
22. Tippe A, Müller-Mohnssen H. Further experimental evidence for the synapse hypothesis of Na^+-current activation and inactivation at the Ranvier node. Naturwissenschaften 1975;62:490-491.
23. Müller-Mohnssen H, Tippe A, Hillenkamp F, Unsöld E. Is the rise of the action potential of the Ranvier node controlled by a paranodal organ? Naturwissenschaften 1974;61:369-370.
24. Dehaene I, Martin JJ, Geens K, Cras P. Guillain-Barré syndrome with ophthalmoplegia: clinicopathologic study of the central and peripheral nervous systems, including the oculomotor nerves. Neurology 1986;36:851-854.

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A. Chiba, S. Kusunoki, H. Obata, R. Machinami and I. Kanazawa
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Q J Med 2002; **95**:717–721

Review

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Treatment of Guillain-Barré syndrome

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Introduction

Although there are earlier clinical descriptions of rapidly progressive weakness that may well have been cases of acute inflammatory neuropathy, Guillain, Barré and Strohl in 1916[1] were the first to demonstrate the peripheral nature of Guillain-Barré syndrome (GBS) by careful recording and interpretation of the tendon reflexes, and thus justified their inclusion in its name. Guillain and his colleagues treated their two patients with bed rest and injections of strychnine, a common treatment at the time. With better understanding of the pathophysiology of the disease and the benefit of controlled trials of a variety of treatments we now have a much firmer evidence base for therapy. This review attempts to summarize and analyse this evidence base.

GBS is an acute neuropathy. Diagnostic criteria were defined for research purposes back in 1981[2] and have been subsequently refined.[3] Essentially, diagnosis requires progressive weakness of more than one limb, over a period of <4 weeks, thought to be due to a neuropathy, in the absence of any identifiable genetic, metabolic or toxic cause. By this definition, GBS is a clinical syndrome that includes a number of pathological and electrophysiological subtypes (Table 1).

The most common and least well understood entity is acute inflammatory demyelinating polyneuropathy (AIDP) that probably constitutes about 75% of the syndrome. Careful neurophysiological assessment will usually show a demyelinating neuropathy. Although not usually required for diagnosis, histological study of nerve at biopsy or post mortem material reveals perivascular infiltrates and demyelination.[4] Although motor findings are by far the most prevalent, the disorder does involve sensory nerves.

Table 1 Clinical subtypes of Guillain-Barré syndrome

Acute inflammatory demyelinating polyneuropathy (AIDP)
Acute motor axonal neuropathy (AMAN)
Acute motor and sensory axonal neuropathy (AMSAN)

Acute motor axonal neuropathy (AMAN) is an axonal, entirely motor disorder which is commonly associated with antibodies against gangliosides, especially GM1.[5] A further type of GBS is the rarer Acute Motor and Axonal Neuropathy (AMSAN), in which neurophysiology and histological findings indicate an acute axonal disorder with involvement of both motor and sensory nerves.[6] A related condition, usually considered to be variant of GBS, is the Miller Fisher syndrome, which was originally coined to describe a condition in which there was ophthalmoplegia, ataxia and areflexia, but no weakness.[7] This disorder is very tightly associated with antibodies to the ganglioside GQ1b, which appears to act in part on the neuromuscular junction to interfere with transmitter release.[8]

The pathogenesis of GBS is only poorly understood. It seems likely that an antibody-mediated mechanism is largely responsible for AMAN and Miller Fisher syndrome, while cellular mechanisms may be more important in AIDP, although antibodies are probably involved in the mechanism of demyelination.

Treatment of GBS can be subdivided into techniques for managing the severely paralysed patient requiring intensive care and respiratory support, and the specific therapy aimed at ameliorating or reversing the nerve damage.

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Supportive management

The advent of respiratory assistance, together with improved intensive care, has improved the outcome of GBS dramatically. While none of these techniques have (or could have been) subjected to controlled trials, the prognosis for GBS has improved such that mortality even in the most severe patients has fallen from 30% to 5%. This is largely due to positive pressure ventilation, but complications of prolonged paralysis can also now be anticipated and appropriate prophylaxis instigated. Thus prophylaxis of venous thrombosis and pulmonary emboli with low-molecular-weight heparin has become routine. Respiratory infections can be reduced by minimal sedation in the ITU, frequent physiotherapy and where appropriate, ventilation with end expiratory pressure to reduce atelectasis and so-called 'elephant lung'. Cardiac rhythm disturbances were a common cause of death in patients with GBS, and this can be reduced by careful ECG monitoring with prophylactic temporary pacing in cases of significant bradycardia, which may herald intractible cardiac asystole. Pain can be adequately controlled with analgesia, and helped greatly by frequent passive limb movements.

Table 2 Disability scale for Guillain-Barré syndrome[28]

Score	Description
0	Healthy
1	Minor symptoms or signs of neuropathy but capable of manual work.
2	Able to walk without support of a stick but incapable of manual work
3	Able to walk with a stick, appliance or support
4	Confined to bed or chairbound
5	Requiring assisted ventilation
6	Dead

Active treatment of GBS

In view of the evidence of immune dysregulation in GBS, treatment with steroids, plasma exchange, and intravenous immunoglobulin have all been tried and reported in the neurological literature. These reports have all initially appeared as anecdotal accounts followed by retrospective series from individual centres. Such data are always difficult to interpret, and a number of randomized controlled trials followed. Such trials have not usually been placebo-controlled because of ethical constraints with sham plasma exchange, but randomization and blinding of the assessors have considerably improved the value of this evidence in making treatment decisions.

Controlled clinical trials have now been carried to assess the value of steroids, plasma exchange, intravenous immunoglobulin and combinations of these treatments. The Cochrane Neuromuscular Group have produced systematic reviews of the evidence supporting the use of each of these treatments.[9–11]

Steroids

Six eligible trials have addressed the value of steroids in treating acute GBS.[12,13] These involved 195 patients and 187 controls. A six-point scale provides the functional endpoint of these trials (Table 2). One large multicentre trial carries most weight in these analyses. Neither mean disability at 4 weeks, (Figure 1) the proportion of patients who were improved by one grade at 4 weeks, nor the improvement in grade at 12 months were altered by steroids, which appear to be safe but ineffective. This contrasts with the treatment of patients with more chronic demyelinating neuropathies, which respond well to steroids. This lack of response to steroids is not easily explained, and it may be that any benefit that steroids have in reducing inflammation is outweighed by some other untoward effect on repair processes. A single pilot study

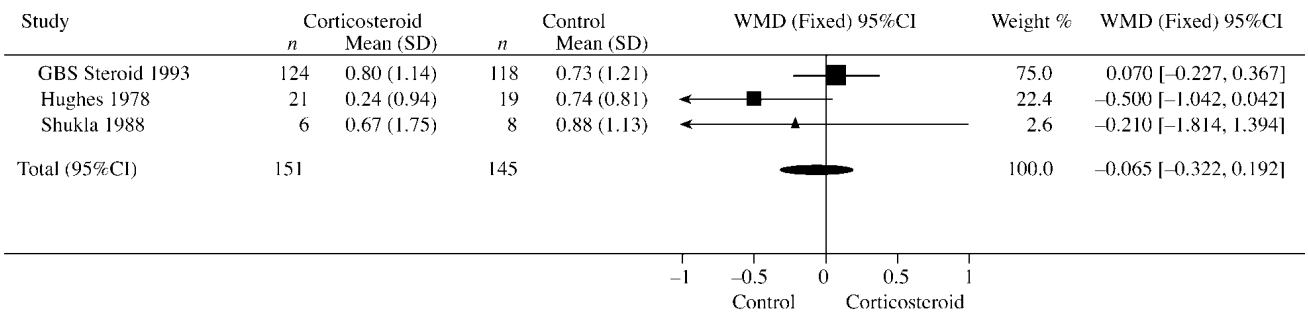

Figure 1. Clinical trials of steroids in Guillain-Barré syndrome. Mean disability grade at 4 weeks.

addressing combined treatment with methyl prednisolone and intravenous immunoglobulin was not included in the Cochrane analysis because it was not randomized, but suggested a possible advantage. A randomized study has recently been presented but not yet published[14] that just fails to find an significant advantage to the combination. *Post hoc* manipulation of the data for known risk factors does suggest an advantage to combination therapy, but such analyses are known to be rather unreliable and can be misleading.

Plasma exchange

The value of plasma exchange has been addressed in six randomized studies, again reviewed by the Cochrane collaboration.[15–20] Overall, 649 patients received plasma exchange, and these were compared with supportive treatment alone, since PE was the first treatment shown to be effective in GBS (Figure 2). The time to recover to walking with aid was significantly shortened in the PE group in two trials (30 vs. 44 days, $p<0.01$). The number of patients that improved one or more grades was available in five trials and gave a relative risk of 1.7 (95%CI 1.42–2.03, $p<0.00001$) in favour of plasma exchange. Similarly there was significant improvements in time to recover walking without aid, percentage of patients requiring artificial ventilation, duration of ventilation, and severe sequelae at one year. The Cochrane review points out that PE is the only treatment for GBS found to be superior to supportive treatment, and therefore should be the standard against which new treatments such as intravenous immunoglobulin should be judged.[19,20]

Two trials have compared the number of different exchanges required for a beneficial effect. Some 304 patients unable to stand unaided were randomized to either four or two exchanges. The median time to recover walking with assistance was significantly shortened in the group receiving four exchanges compared to two exchanges (24 vs. 20 days, $p=0.04$). Significant shortening was also seen in this group in the median time on the ventilator and the median time to hospital discharge. At 12 months, there was also a higher proportion of patients that had recovered full muscle strength among those treated with four exchanges. This benefit of four rather two exchanges was accompanied by a slightly increased incidence of blood pressure instability and haematomas, although these were not considered sufficiently severe to outweigh the advantage of the extra exchanges. In a second trial the same authors examined six versus four exchanges in patients whose disease was severe enough to require mechanical ventilation at entry. No difference in efficacy was observed between these two groups, but adverse events were more frequent in the six-exchange group, with significantly greater blood pressure instability in this group (46% vs. 26%, $p=0.001$) and slightly more deaths in this group at one year (4/80, 5% vs. 2/81, 2%; $p=0.44$. From this data it appears that four exchanges are better than two for moderate disease, and two sessions suffice for patients with only mild disease.

PE has also been compared to CSF fluid filtration in a single randomized trial.[21] In this trial, 20 patients were assigned to PE (five or six sessions) and compared with 17 patients treated with CSF filtration. The CSF filtration consisted of five or six cycles of 30 to 50 ml of CSF filtered and reinstilled daily for 15 days. Median improvement of clinical grades was not significantly different at four weeks, nor was there any significant advantage to CSF

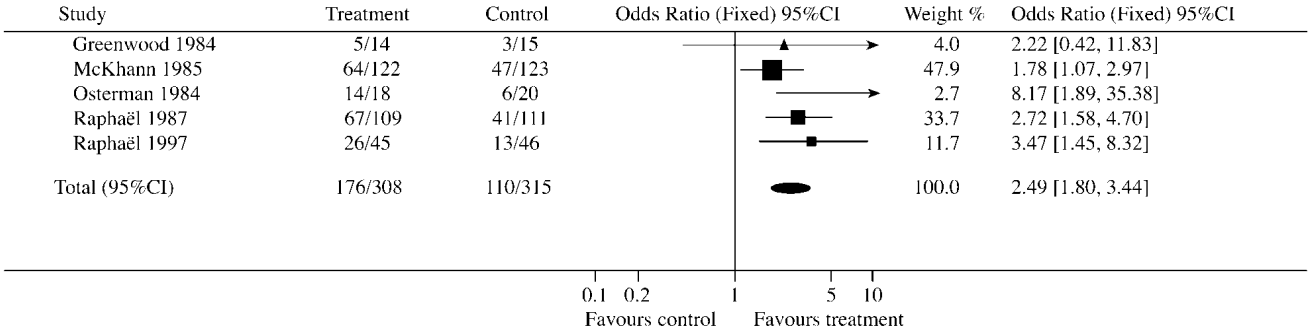

Figure 2. Plasma exchange in Guillain-Barré syndrome. Proportion of patients improving one grade at 4 weeks. From the Cochrane Library,[9] reproduced with permission of Update Software. Cochrane reviews are regularly updated as new information becomes available and in response to comments and criticisms. The reader should consult The Cochrane Library for the latest version of a Cochrane Review. Information on The Cochrane Library can be found at [www.update-software.com].

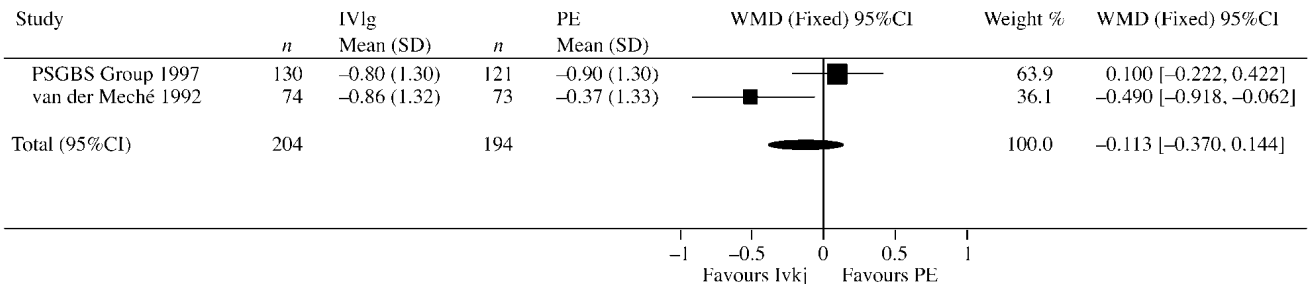

Figure 3. Intravenous immunoglobulin vs. plasma exchange in Guillain-Barré syndrome. Change in disability grade at 4 weeks.

filtration. This trial was quite small, so clear conclusions on the value of CSF filtration cannot be made, but as yet there seems no good evidence to use this treatment.

Intravenous immunoglobulin

Intravenous immunoglobulin (IVIg) was introduced for the treatment of auto-immune thrombocytopaenia[22] and tried for the treatment of chronic inflammatory demyelinating polyneuropathy.[23] A favourable response in patients with GBS was reported in 1988[24] and led to the first randomized controlled trial. A meta analysis of IVIg for GBS found three randomized trials that compared IVIg with PE[25,26] and the only trial comparing IvIg with supportive treatment was considered inadequate to establish its value.[27]

IVIg appeared beneficial in all three randomized controlled trials (Figure 3). Two of these trials could be combined in a meta-analysis to give 398 patients, and the value of IVIg assessed on the basis of a change in disability scale employed in previous studies. There was no significant difference between IVIg and PE in change of disability grade, nor in time to walk unaided, mortality, or proportion of patients unable to walk at one year.

Conclusion

Good quality intensive care remains the most important aspect of the management of the severe case of GBS. Clinical trials indicate that plasma exchange is more effective than supportive treatment alone in reducing the median time taken for patients to recover. Intravenous immunoglobulin appears as effective as plasma exchange and may have fewer side-effects. Corticosteroids alone do not alter the outcome of GBS, and there is insufficient evidence that their use in combination with immunoglobulin is effective. Other treatments such as CSF filtration remain experimental and unproven.

References

1. Guillain G, Barré JA, Strohl A. Sur un syndrome de radiculo-nevrite avec hyperalbuminose du liquide cephalorachidien sans reaction cellulaire: remarques sur les characteres clinique et graphique des reflexes tendinaux. *Bull Soc Med Hop* 1916; **40**:1462–70.
2. Asbury AK, Arnason BG, Karp HR, McFarlin DE. Criteria for the diagnosis of Guillain-Barré syndrome. *Ann Neurol* 1978; **3**:565–6.
3. Asbury AK, Cornblath DR. Assessment of current diagnostic criteria for Guillain-Barré syndrome. *Ann Neurol* 1990; **27**:S21–4.
4. Asbury AK, Arnason BG, Adams RD. The inflammatory lesion in idiopathic polyneuritis: its role in pathogenesis. *Medicine* 1969; **48**:173–215.
5. Ho TW, Mishu B, Li CY, *et al.* Guillain-Barré syndrome in northern China: relationship to *Campylobacter jejuni* infection and anti-glycolipid antibodies. *Brain* 1995; **118**:597–605.
6. Hughes R, Hadden R, Gregson N, Smith K. Pathogenesis of Guillain-Barré syndrome. *J Neuroimmunol* 1999; **100**:74–97.
7. Fisher C. An unusual variant of acute idiopathic polyneuritis (syndrome of ophthalmoplegia, ataxia and areflexia). *N Engl J Med* 1956; **225**:57–75.
8. Chiba A, Kusunoki S, Obata H, Machinami R, Kanazawa I. Serum anti-GQ1b IgG antibody is associated with ophthalmoplegia in Miller Fisher syndrome and Guillain-Barré syndrome: clinical and immunohistochemical studies. *Neurology* 1993; **43**:1911–17.
9. Hughes RAC, van der Meché FGA. *Corticosteroid Treatment for Guillain-Barré Syndrome (Cochrane Review).* Oxford, Update Software, 1999.
10. Hughes RAC, Raphael JC, Swan AV, van Doorn PA. *Intravenous Immunoglobulin for Guillain-Barré Syndrome (Cochrane Review).* Oxford, Update Software, 2002.
11. Raphael JC, Chevret S, Hughes RAC. *Plasma Exchange for Guillain-Barré Syndrome (Cochrane Review).* Oxford, Update Software, 2001.
12. Anonymous. Double-blind trial of intravenous methylprednisolone in Guillain-Barré syndrome: Guillain-Barré Syndrome Steroid Trial Group. *Lancet* 1993; **341**:586–90.

13. Swick HM, McQuillen MP. The use of steroids in the treatment of idiopathic polyneuritis. *Neurology* 1976; **26**:205–12.
14. Van Koningsveld R, van der Meché FGA, Schmitz PI, Van Doorn PA. Combined therapy of intravenous immunoglobulin and methylprednisolone in patients with Guillain-Barré syndrome: the results of a multicentre double blind placebo controlled clinical trial. *Peripher Nerv Syst* 2001; **6**:186–7.
15. Farkkila M, Kinnunen E, Haapanen E, Iivanainen M. Guillain-Barré syndrome: quantitative measurement of plasma exchange therapy. *Neurology* 1987; **37**:837–40.
16. Greenwood RJ, Newsom-Davis J, Hughes RA, *et al.* Controlled trial of plasma exchange in acute inflammatory polyradiculoneuropathy. *Lancet* 1984; **1**:877–9.
17. Anonymous. Plasmapheresis and acute Guillain-Barré syndrome: the Guillain-Barré syndrome Study Group. *Neurology* 1985; **35**:1096–104.
18. Osterman PO, Fagius J, Lundemo G, *et al.* Beneficial effects of plasma exchange in acute inflammatory polyradiculoneuropathy. *Lancet* 1984; **2**:1296–9.
19. Anonymous. Efficiency of plasma exchange in Guillain-Barré syndrome: role of replacement fluids. French Cooperative Group on Plasma Exchange in Guillain-Barré syndrome. *Ann Neurol* 1987; **22**:753–61.
20. Anonymous. Appropriate number of plasma exchanges in Guillain-Barré syndrome: French Cooperative Group on Plasma Exchange in Guillain-Barré Syndrome. *Ann Neurol* 1997; **41**:298–306.
21. Wollinsky K, Hulser P, Brinkmeier H, *et al.* CSF filtration is an effective treatment of Guillain-Barré syndrome: a randomized clinical trial. *Neurology* 2001; **57**:774–80.
22. Imbach P, Barundun S, d'Apuzzo V, Baumgartner C, Hirt A, Morell A, *et al.* High dose intravenous gammaglobulin for idiopathic thrombocytopaenic purpura. *Lancet* 1981; **1**:1228–31.
23. Vermeulin M, van der Meché FGA, Speelman JD, Weber A, Busch HFM. Plasma and gammaglobulin infusion in chronic inflammatory polyneuropathy. *J Neurol Sci* 1985; **70**:317–26.
24. Kleyweg RP, van der Meché FGA. Treatment related fluctuations in Guillain-Barré syndrome after high-dose immunoglobulins or plasma-exchange. *J Neurol Neurosurg Psychiat* 1991; **54**:957–60.
25. van der Meché FGA, Schmitz PI. A randomized trial comparing intravenous immune globulin and plasma exchange in Guillain-Barré syndrome. Dutch Guillain-Barré Study Group. *N Engl J Med* 1992; **326**:1123–9.
26. Anonymous. Randomised trial of plasma exchange, intravenous immunoglobulin, and combined treatments in Guillain-Barré syndrome: Plasma Exchange/Sandoglobulin Guillain-Barré Syndrome Trial Group. *Lancet* 1997; **349**:225–30.
27. Gurses N, Uysal S, Cetinkaya F, Islek I, Kalayci AG. Intravenous immunoglobulin treatment in children with Guillain-Barré syndrome. *Scand J Infect Dis* 1995; **27**:241–3.
28. Hughes RAC, Newsom-Davis JM, Perkin GD, Pierce JM. Controlled trial of prednisolone in acute polyneuropathy. *Lancet* 1978; **2**:750–3.

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Transgenic and knockout databases: Behavioral profiles of mouse mutants

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Abstract

Genetically engineered strains of mice, modified by transgenesis or gene targeting ("knockouts") are being generated at an impressive rate and used, among other areas, as premiere research tools in deciphering the genetic basis of behavior. As behavioral phenotyping strategies continue to evolve, characterization of these "designer" mice will provide models to evaluate the efficacy of new pharmacological and gene therapy treatments in human hereditary diseases. Reported behavioral profiles include aberrant social, reproductive, and parental behaviors, learning and memory deficits, feeding disorders, aggression, anxiety-related behaviors, pain/analgesia, and altered responses to antidepressants, antipsychotics, ethanol, and psychostimulant drugs of abuse. The Induced Mutant Resource (IMR) at The Jackson Laboratory (TJL, Bar Harbor, ME, USA) imports, cryopreserves, develops, maintains, and distributes biomedically important stocks of transgenic and targeted mutant mice to the research community. Information on neurological/behavioral strains — including behavioral performance, husbandry requirements, strain availability, and genetic typing protocols — is provided through the IMR database (http://www.jax.org/resources/documents/imr/). A catalog of available strains is readily accessible via the JAX® Mice website at http://jaxmice.jax.org/index.shtml. In addition, TJL is now host to TBASE (http://tbase.jax.org/), a comprehensive, public-domain database with primary emphasis on mouse knockouts. TBASE contains an exhaustive list of knockout-related citations and provides an extensive phenotypic characterization of numerous behavioral mutants that is extracted directly from the literature. Present efforts to merge the two resources into a novel, schematically enhanced database, provisionally named Transgenic and Targeted Mutation Database (TTMD), will be briefly discussed. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Database; Transgenic; Knockout; Mice; Phenotype; Behavior; Strain availability; IMR; TBASE

1. Introduction

Recent advances in experimental manipulation of the mouse via transgenesis and gene targeting ("knockout" technology) have provided powerful probes into sophisticated biological systems such as gene interaction and molecular regulation of complex neurophysiology and behavior. Traditionally, genetic approaches toward analyzing mouse behavior have focused on preexisting, "natural" genetic differences amongst laboratory inbred strains. Although the development of quantitative trait loci (QTL) methodology using recombinant inbred strains, has extended genetic analysis to the more common complex traits diseases, the molecular identification of the gene(s) involved in complex behavioral and drug–response traits is still a major challenge. Targeted germline mutations offer premiere research tools for deciphering the genetic basis of mammalian behavior. As more and more technological limitations are circumvented, these sophisticated animal mutants provide alternative means for testing pharmacological interventions, evaluating constructs for gene therapy and identifying modifier loci that dictate phenotypic severity. A number of excellent reviews have been published on behavioral transgenic and knockout mice, addressing the latest developments in experimental design and phenotyping strategies [8,14,17,36,43,64,65,72,73,90,98]. Many of them emphasize the need for extensive knowledge of the endogenous traits of inbred mouse strains, and the importance of selecting an optimal inbred background that is least expected to compromise the interpretation of the mutant phenotype in a given behavioral task.

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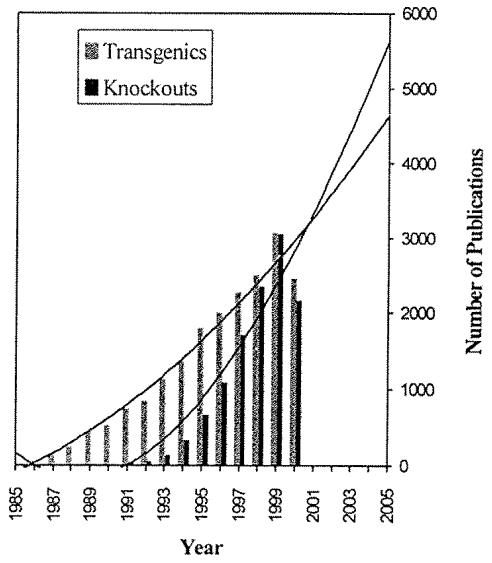

Fig. 1. Literature citation trends for transgenic and targeted mutant mice ("knockouts"). Current values (1985–August 15, 2000) were obtained by searching Medline. Projected values (August 15, 2000–2005) were generated utilizing historical rates and trends for transgenic and knockout publications. Polynomial regression trendlines in this chart represent two orders of freedom.

Considering the hundreds of known genes now amenable to gene targeting (Fig. 1), it is not surprising to see an extremely rapid increase in the number of mouse mutant strains with behavioral manifestations. Accordingly, a spectacular number of knockouts have been generated for genes expressed in the central nervous system (CNS) (Fig. 2). Behavioral neuroscientists are currently analyzing the phenotypic profiles of these extraordinary mice (Table 1). The emergence of conditional knockouts designed to restrict the effects of a given mutation to a specific developmental stage and/or one cell type using Cre/LoxP-mediated recombination will undoubtedly have a considerable impact on the data growth and future direction of behavioral genetics. Indeed, one limitation to these studies may be the logistics of maintaining the large number of mouse strains that will be generated from targeted embryonic stem (ES) cells, and from interbreeding targeted strains with one another, with existing transgenic mice, and with naturally occurring mutant strains. The very success of these genetic manipulations has raised important issues pertaining to the access and availability of popular knockout and transgenic animals, as well as the availability of Internet resources designed for the storage and organization of experimental data about them [5].

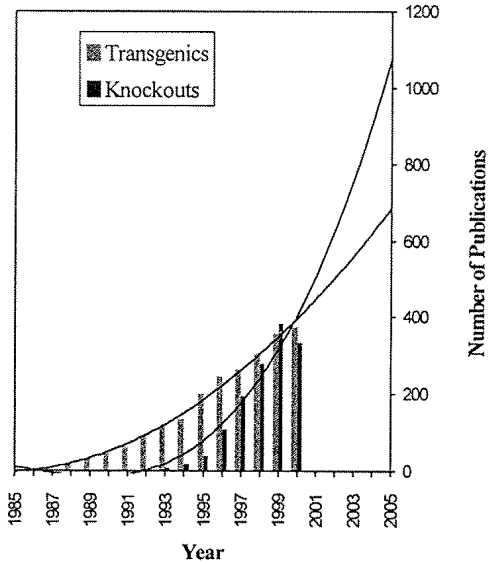

Fig. 2. Literature citation trends for CNS-related transgenic and targeted mutant mice ("knockouts"). Current values (1985–August 15, 2000) were obtained by searching Medline. Projected values (August 15, 2000–2005) were generated utilizing historical rates and trends for CNS-related transgenic and knockout publications. Polynomial regression trendlines in this chart represent two orders of freedom.

Table 1
The number of publications citing either transgenic or knockout mice listed by behavioral phenotype[a]

Behavioral phenotype	Transgenic mice	Knockout mice
Aggression	29	39
Alcohol response	279	199
Anxiety	32	61
Despair	2	1
Epilepsy	51	69
Fear	21	35
Feeding	21	39
Learning	189	238
Maternal behavior	8	22
Memory	361	234
Motor capabilities	445	298
Pain	38	92
Stress	336	314

[a] Current values (1985–August 15, 2000) were obtained by searching Medline and include review articles.

Internet resources dedicated to the accumulation and dissemination of data on transgenic and knockout mouse models at The Jackson Laboratory (TJL) are presented in Table 2. The general scope, contents, and search capabilities of each resource are discussed in the following sections in relevance to mouse behavioral profiles. Additional Internet resources describing murine behavioral phenotypes are recommended in Table 3. The majority of these sites do not require subscription and represent community databases focusing on specialized methodologies including Cre/LoxP-mediated recombination, gene trap

Table 2
Transgenic and knockout Internet resources hosted at TJL

Internet resource	Home page address
IMR	http://www.jax.org/resources/documents/imr/
JAX® Mice	http://jaxmice.jax.org/index.shtml
JAX® GEMM™ Strains	http://jaxmice.jax.org/html/pricelist/jaxgemmstrains.shtml
TBASE	http://tbase.jax.org/

Table 3
Additional transgenic and knockout databases listing behavioral phenotypes

Transgenic/knockout database	Home page address
BioMedNet Mouse Knockout Database	http://research.bmn.com/mkmd
Cre Transgenic and Floxed Gene Database	http://www.mshri.on.ca/nagy/cre.htm
Database of Gene Knockouts	http://www.bioscience.org/knockout/knochome.htm
Sinai's Mammary Transgene Database	http://mbcr.bcm.tmc.edu:80/BEP/ERMB/mtdb.html
Skarnes Resource of Gene Trap Insertions	http://socrates.berkeley.edu/~skarnes/resource.html
The Big Blue website	http://eden.ceh.uvic.ca/bigblue.htm
The Whole Mouse Catalog	http://www.rodentia.com/wmc/

insertions, and genomic integration of the bacterial lacZ or lacI genes.

2. Availability and phenotypic characterization of behavioral mouse mutants at TJL

2.1. The Induced Mutant Resource

The Induced Mutant Resource (IMR) at TJL was established in 1992 and serves as the US National clearing house for the collection and distribution of genetically engineered strains of mice. These include biomedically valuable strains of mice altered by transgenesis, gene targeting, and chemical mutagenesis [82,83]. The function of the IMR is to (1) select stocks of transgenic, targeted, and chemically mutagenized mice in high demand, (2) import these stocks into TJL by rederivation procedures that free them of any pathogens that they might harbor, (3) cryopreserve embryos from these stocks to ensure protection against accidental damage and genetic contamination, (4) backcross the mutation onto an inbred strain, if required, and (5) distribute them to the scientific community. Information collected about mutants considered for admission is presented to an internal Laboratory Committee, The Genetic Resources Committee. The ultimate decision on accepting a candidate strain rests with this committee, which meets on a monthly basis. Criteria for selecting mutants are based on existing guidelines for importing mice to the laboratory's Genetic Resources. These criteria include: (a) the immediate demand for use in biomedical research, (b) the numbers of requests for animals being received by the original investigators, (c) the potential for future research, (d) the time and effort needed to replace or recreate the mutant or strain; finally, (e) the uniqueness of the "designer" mutation or strain. Potential IMR strains are identified by direct submissions from their original creators, recommendations from the IMR Advisory Board or Associated Board Members, suggestions from scientists, and from literature searches.

As this paper went to press, the IMR had accepted a cumulative total of 737 strains, with over 495 of these currently available, 49 undergoing rederivation, and 112 available from frozen embryos. The IMR currently accepts approximately seven new strains each month. The average time to level 4 distribution ("available as breeder pairs or individual mice") is approximately 7 months following strain arrival at the Importation Facility. Circumstances such as the number of animals initially received for the hysterectomy derivation process, the genotype of these animals, as well as their breeding capacity in isolators, determines the actual time to reach distribution.

The IMR maintains an accompanying online database to provide information about these strains (Table 2). In particular, the IMR database stores data on 96 neurological/behavioral mouse models: of these, two have been generated by chemical mutagenesis, 11 by transgenesis, and 83 by homologous recombination in ES cells, with several strains distributed on more than one genetic background. Information furnished about each strain includes stock number, gene symbol and name, chromosomal location, genetic background, and primary reference(s). A link to Mouse Genome Database (MGD, http://www.informatics.jax.org/) is also provided for each mutant. Additional information encompasses: genetic typing protocols; information data sheets, including a synopsis of behavioral performance; husbandry requirements; guidelines on licensing and patent restrictions, if applicable; access to technical support via micetech@jax.org; and, finally, electronic forms for direct strain submission.

A current catalog of available strains is readily accessible via the JAX® Mice website (Table 2). Among other useful links, this site provides a price list and complete product guide related to selecting, ordering, and using JAX® Mice. Specifically, Genetically Engineered and Mutant Mice (JAX® GEMM™ Strains; Table 2) are searchable by mutation type (transgenic, targeted, or chemically induced). In addition, mouse models that are relevant to neurobiology research are listed by behavioral phenotype at http://jaxmice.jax.org/jaxmicedb/html/sbmodel_13.shtml. As always, strain availability information is accompanied by stock number, official transgenic or knockout strain nomenclature, genetic background, and standard supply data, as well as pricing and gender/genetic typing details.

2.2. The database of transgenic animals and targeted mutations (TBASE)

TBASE is the original community database that maintains comprehensive and current coverage of all transgenic animals and targeted mutants generated worldwide. Originally

existing as a flat file database [99], TBASE was successfully adopted by and released as a World Wide Web interface from the Division of Biomedical Information Sciences at The Johns Hopkins University in early 1994. Details on the implementation of its relational schematic design have been reported elsewhere [5]. Presently hosted at TJL (Table 2), TBASE continues to serve as a dynamic, central repository that ensures free, systematic access and direct communication of data on transgenesis and gene targeting [37,63,99]. Because the human genome initiative has accentuated the significance of the mouse as the predominant mammalian model system, special emphasis has been placed on mouse knockouts. Spontaneous and chemically induced mutants are presently excluded from the database.

At its fundamental level, TBASE stores the exploding data on the production and characterization of transgenic animals and targeted mutants. It contains information pertaining to the experimental methodologies and techniques used to produce them, and phenotypic attributes, as well as any applications that they may have in specific research fields. In contrast to the IMR database, TBASE is predominantly populated by literature scanning, that is, direct data extraction from the scientific literature through regular examination of relevant journals. Active literature scanning continues to be the primary mode of data accumulation, and ensures that the database faithfully reflects the general direction of research on transgenesis and gene targeting. Following selection of material, pertinent data are then manually entered in the database. In an effort to avoid missing important data, keyword-related references from specialized journals are periodically screened. Relevant references are loaded directly from Medline into an autonomous citation database (TBASE CitDB), which is updated on a weekly basis and ultimately represents an exhaustive bibliographical resource for transgenic and knockout experimentation. Importantly, TBASE accounts for both published and unpublished material, so that data that are unavailable in the literature may be accessed, and duplication of time-consuming and costly experiments can be avoided. Unpublished material is entered in the database as a "personal communication" with a specified contact person. Inclusion of such information is considered a public disclosure, and TBASE can be cited as a source when referencing a novel mutant not described in the literature [37,99].

Importantly, TBASE provides an extensive, detailed phenotypic characterization of mouse mutants, accommodating subtle phenotypic alterations in addition to prominent or fully penetrant abnormalities. Experimental design, evaluation of general health, sensory and motor functions, as well as descriptions of specific behavioral paradigms are incorporated, if noted in the original article. Furthermore, TBASE presents conclusive remarks and provides direct reference to the designated corresponding author, who may furnish details about generating or acquiring a maintained strain not available through the IMR. (So far, information on maintenance and availability of mutant mouse stains has been generally scarce in TBASE, as it does not appear systematically in the literature.) In addition, the TBASE database describes "related" mutant strains (parental and derived), as in the case of "multiple" transgenics or knockouts, and incorporates an "Application" field that briefly describes any relevance for therapeutic or research value. Notably, all mutants heterozygous for a given targeted allele are cataloged independently of their homozygous null counterparts, since they often display remarkable phenotypes. To illustrate, mice heterozygous for a *Nf1* (neurofibromatosis type 1) targeted allele (TBASE:4217) exhibit learning and memory deficits that are restricted to specific types of learning, are not fully penetrant, and do not involve deficits in simple associative learning [85]; *Emx1* heterozygotes (TBASE:3950) show partial penetrance for the corpus callosum abnormality encountered in *Emx1*-null mice (TBASE:3951) [75]; finally, loss of neurotrophin-3 leads to significant retardation in spontaneous hair follicle regression (catagen) in newborn *Ntf3* heterozygous mutants [20b].

Two additional TBASE features highlight the importance of cataloging knockout mice as putative models for human genetic disorders. The first one entitled *It's a Knockout!* is a quarterly column that provides a concise phenotypic profile of the latest mouse knockouts (http://tbase.jax.org/docs/knockout.html). Each column is in fact an electronic adjunct of a quarterly printed contribution to *Trends in Genetics* [37]. The second feature entitled *Knockout Model of the Month* is a monthly report describing a new mutant with an unusual or previously unsuspected phenotype (http://tbase.jax.org/docs/monthly.html). Both features contain selected bibliography and numerous links to related electronic sites as well as to genomic or specialized databases including IMR, MGD, The National Center for Biotechnology Information (NCBI) Locus Link (http://www.ncbi.nlm.nih.gov/LocusLink/index.html), Online Mendelian Inheritance in Man (OMIM; http://www3.ncbi.nlm.nih.gov/omim/), Genome Database (GDB, http://gdbwww.gdb.org/), and GeneCard (http://bioinfo.weizmann.ac.il/cards/). Behavioral and neurological targeted mutants that have been presented in this section include: the *Htr1b* [5-hydroxytryptamine (serotonin) receptor 1B] knockouts (TBASE:3922) shown to display aggression and reduced sensitivity to ethanol-induced ataxia (http://tbase.jax.org/docs/serotonin.html); the *Otx1* (orthodenticle homolog 1 homeobox) knockouts (TBASE:4075) displaying spontaneous seizures and brain malformations (http://tbase.jax.org/docs/epilepsy.html); the *Fmr1* (fragile X mental retardation syndrome 1 homolog) knockouts (TBASE:4389) exhibiting altered dendritic spine morphology and density, as well as the behavioral deficits and macroorchidism noted in the human Fragile X syndrome (http://tbase.jax.org/docs/fragile.html); the socially inept *Dvl1* (dishevelled 1) knockouts (TBASE:4524) showing sensorimotor gating deficits (http://tbase.jax.org/docs/Dvl1.html); and the hyperactive *Dat1* (dopamine transporter 1) knockouts (TBASE:5023)

Table 4
Neurological and behavioral mouse models listed in IMR and TBASE

Gene[a]	IMR strain/stock number	TBASE ID	Reference	Knockout phenotype
Altered responses to antidepressants, antipsychotics, and psychostimulant drugs of abuse				
Acra7	C57BL/6-Acra7^{tm1Bay}/003232	none	[68]	Absence of α-bungarotoxin binding sites and hippocampal fast nicotinic currents.
Gabrd	B6,129-Gabrd^{tm1Geh}/003725	none	[59]	Selective attenuation of responses to neuroactive steroids, but no t to other modulatory drugs. Electrophysiological recordings from hippocampal slices reveal a faster miniature inhibitory postsynaptic current decay time, with no change in miniature inhibitory postsynaptic current amplitude or frequency. See *Learning and memory deficits.*
Drd1a	STOCK Drd1a^{tm1Jcd}/002322	3299, 3300, 3310, 3311	[18]	Retained cocaine conditioned place preference. See *Motor/coordination/balance impairments.*
	C57BL/6J-Drd1a^{tm1Jcd}/002959			
Aberrant social, reproductive, and parental behaviors				
Acvr2a	B6,129-Acvr2^{tm1Zuk}/003277	1949, 1950	[57]	Defective reproductive performance.
Fosb	BALB/c,129-Fosb^{tm1Meg}/003077	3857, 3858	[10]	Profound deficiency in nurturing ability. Normal cognitive and sensory functions.
Gabrb3	B6,129S-Gabrb3^{tm1Geh}/002711	5010	[30a]	Neonatal mortality often accompanied by cleft palate. Surviving mice are fertile but mothers fail to nurture offspring. See *Motor/coordination/balance impairments* and *Seizures.*
Nos1	B6,129S-Nos1^{tm1Pth}/002633	986, 988, 2764, 2765, 2829, 3065, 3634	[35]	Increase in aggressive behavior and excessive, inappropriate sexual behavior. See *Aggression* and *Miscellaneous.*
	C57BL/6J-Nos1^{tm1Pth}/002986			
Sod1	B6,129S-Sod1^{tm1Leb}/002972	none	[56]	Female infertility. Ovaries often display many primary and small antral follicles but few corpora lutea.
Sp4	STOCK Spr^{tm1Ssp}/003119	3767, 3768	[89]	Neonatal death. Surviving homozygous null males do not breed, despite presence of histologically intact testes and mature sperm. Failure to copulate.
Learning and memory deficits				
Apoe	C57BL/6J-Apoe^{tm1Unc}/002052	750, 752, 753, 755, 955, 956, 1163, 5099	[74,94,95]	Altered responses to stress, impaired spatial learning and memory, altered LTP, and synaptic damage.
Camk2a	C57BL/6-Camk2a^{tm1}/002362	2082, 2083, 2507, 2508	[86]	Absence of neuroanatomical defects, normal behavior, and normal postsynaptic mechanisms. Inability to produce LTP: suitable model for studying the relation between LTP and learning processes. See *Seizures.*
Fmr1	FVB,129P-Fmr1^{tm1Cgr}/002700	2631, 2632, 4389, 4826	[92]	Model for Fragile X syndrome: learning deficits, and hyperactivity. See *Miscellaneous.*
	FVB/NJ-Fmr1^{tm1Cgr}/003024			
	C57BL/6J-Fmr1^{tm1Cgr}/003025			
Fos	B6,129X1-Fos^{tm1Pa}/002099	188, 2561, 2562, 3623, 4149	[69]	Impairment of complex learning on the Morris water task. See *Motor/coordination/balance impairments* and *Seizures.*
	B6,129S-Fos^{tm1Pa}/002293			
	STOCK Fos^{tm1Pa}/003286			
Fyn	129-Fyn^{tm1Sor}/002271	none	[25]	Blunted LTP, impaired special learning, and altered hippocampal development.
Gabrd	B6,129-Gabrd^{tm1Geh}/003725	none	[59]	Normal learning and memory assessed with fear conditioning. See *Altered responses to drugs of abuse.*
Gprc1d	STOCK Gprc1d^{tm1Hpn}/003576	none	[24]	Altered spatial learning and memory.
Gria2	STOCK Gria2^{tm1Rod}/002913	none	[38]	Enhanced and nonsaturating LTP in the CA1 region of hippocampus, with high increase in $Ca2^+$ permeability in response to kainate application. Normal neuronal excit ability and paired-pulse facilitation. See *Motor/coordination/balance impairments.*
	C57BL/6J-Gria2^{tm1Rod}/003143			

(*continued on next page*)

Table 4 (*continued*)

Gene[a]	IMR strain/stock number	TBASE ID	Reference	Knockout phenotype
Grm4	STOCK Grm4^{tm1Hpn}/003576	none	[70]	Inability to learn complex motor tasks. Impaired paired-pulse facilitation and posttetanic potentiation. Normal LTD. See *Motor/coordination/balance impairments.*
Grm5	B6,129-Grm5^{tm1Rod}/003121	none	[53]	Significant reduction of LTP in the NMDA receptor-dependent pathways (CA1 region and dentate gyrus of the hippocampus); intact LTP in the mossy fiber synapses on the CA3 region, an NMDAR-independent pathway. Aberrant acquisition and use of spatial information in both the Morris water maze and contextual information in the fear-conditioning test.
Ncam	C57BL/6-Ncam^{tm1Cgn}/002405	1013, 1015	[15]	10% reduction in overall brain weight and a 36% decline in size of olfactory bulb. Deficits in spatial learning (Morris water maze). Normal activity and motor abilities.
Nfl	C57BL/6-Nfl^{tm1Fcr}/002646	4217	[85]	Learning and memory deficits in Nfl heterozygotes are restricted to specific types of learning, they are not fully penetrant, and can be compensated for with extended training. No deficits in simple associative learning. See *Miscellaneous.*
Pkcc	B6,129P-Pkcc^{tm1Stl}/002466	1050, 1052, 1056, 1058, 4162, 4650	[2]	Modified LTP of synaptic transmission in the hippocampus. Normal LTD and paired-pulse facilitation. Mild deficits in spatial and contextual learning. See *Response to ethanol* and *Motor/coordination/balance impairments.*
Rab3a	B6,129S-Rab3a^{tm1Sud}/002443	3267, 3269, 5480	[23a]	No alterations in a variety of short-term plasticities. Abolished LTP at mossy fiber synapses.
Syn1	B6,129S-Syn1^{tm1Sud}/002444	none	[78]	Selective increase in paired pulse facilitation. No alterations in other synaptic parameters such as LTP. The number of vesicles exocytosed during brief action potential trains and the total recycling vesicle pool are significantly reduced.
Syn2	B6,129S-Syn2^{tm1Sud}/002477	none	[77]	Decreased posttetanic potentiation and severe synaptic depression upon repetitive stimulation. Intrinsic synaptic vesicle membrane proteins, but not peripheral membrane proteins or other synaptic proteins are slightly decreased.
Sty1	B6,129S-Syt1^{tm1Sud}/002478	none	[23b]	Neonatal death. The synchronous, fast component of Ca^{2+}-dependent neurotransmitter release is decreased, whereas asynchronous release processes, including spontaneous synaptic activity (miniature excitatory postsynaptic current frequency) and release triggered by hypertonic solution or α-latrotoxin, are unaffected.
Feeding disorders				
Grpr	B6,129X-Grpr^{tm1Jfb}/003126	none	[27]	Loss of bombesin-induced feeding suppression.
Htr2c	C57BL/6-Htr2c^{tm1Jul}/002627	4112, 5482	[91]	Increased weight as a result of abnormal control of feeding behavior. See *Seizures.*
Ntrk2	B6,129S-Ntrk2^{tm1Bbd}/002544	1023, 1025	[45]	Absence of feeding activity (sucking pattern). Neonatal death. See *Miscellaneous.*
	C57BL/6J-Ntrk2^{tm1Bbd}/003098			
Aggression				
Nos1	B6,129S-Nos1^{tm1Pth}/002633	986, 988, 2764, 2765, 2829, 3065, 3634	[35]	Increase in aggressive behavior and excessive inappropriate sexual behavior. See *Aberrant social, reproductive, and parental behaviors* and *Miscellaneous.*
	C57BL/6J-Nos1^{tm1Pth}/002986			
Anxiety-related behaviors				
Drd3	B6,129S-Drd3^{tm1Dac}/002425	none	[3]	Evidence for reduced anxiety in the open-field and the elevated plus-maze test. See *Motor/coordination/balance impairments.*
	C57BL/6J-Drd3^{tm1Dac}/002958			

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Table 4 (*continued*)

Gene[a]	IMR strain/stock number	TBASE ID	Reference	Knockout phenotype
Pain/analgesia				
Pomc	C57BL/6-Pomc^{tm1Low}/003191	3562, 3563	[79]	Normal analgesia in response to morphine, indicating the presence of functional mu-opiate receptors. Absence of opioid (naloxone reversible) analgesia induced by mild swim stress. Enhanced nonopioid analgesia in response to cold water swim. Paradoxical naloxone-induced analgesia.
Response to ethanol				
Gabra6	B6,129S-Gabra6^{tm1Geh}/002710	none	[30b]	Normal cerebellar cytoarchitecture. Normal neuronal pathway mediating the hypnotic effect of ethanol and its antagonism by Ro15-4513. Normal behavioral responses to general anesthetics or pentobarbital.
Gabrg2L	B6,129-Gabrg2L^{tm1Geh}/003137	none	[29]	Normal behavioral effects of ethanol: sleep time, anxiolysis, acute functional tolerance, chronic withdrawal hyperexcitability, and hyperlocomotor activity remain unaffected.
Pkcc	B6,129P-Pkcc^{tm1Stl}/002466	1050, 1052, 1056, 1058, 4162, 4650	[2]	Reduced sensitivity to the effects of ethanol on loss of righting reflex and hypothermia. Normal responses to flunitrazepam or pentobarbital. Altered function of γ-aminobutyrate type A receptors. See *Learning and memory deficits* and *Motor/coordination/balance impairments.*
Motor/coordination/balance impairments				
Aga	STOCK Aga^{tm1Vk}/003463	none	[40]	Model for the human lysosomal disease, aspartylglycosaminuria, and impaired neuromotor coordination.
Atf2	C,129-Atf2^{tm1Glm}/002419	3149, 3150	[76]	Gait, hyperactivity, and decreased hearing. Reduced numbers of cerebellar Purkinje cells, atrophic vestibular sense organs, and enlarged ventricles in the brain.
Bdnf	STOCK Bdnf^{tm1Jae}/002267	1149, 1151, 3199	[20a]	Deficiencies in coordination and balance associated with excessive degeneration in several sensory ganglia including the vestibular ganglion; suppressed development of kindling heterozygous null mice. See *Seizures.*
	B6,129S4-Bdnf^{tm1Jae}/002266			
Calb1	B6,129-Calb1^{tm1Moin}/003079	none	[4]	Impaired motor coordination and ataxia. Altered dendritic calcium signaling.
Cstb	129/SvJ-Cstb^{tm1Rm}/003486	5014, 5015	[71]	Ataxia and symptoms reminiscent of the human Unverricht–Lundborg disease. Loss of cerebellar granule cells with features of apoptosis. See *Seizures.*
Drd1a	STOCK Drd1a^{tm1Jcd}/002322	3299, 3300, 3310, 3311	[18]	Normal coordination and locomotion with significant decrease in rearing behavior. Selective functional alterations in the striatal neurons giving rise to the direct striatal output pathway. See *Altered responses to drugs of abuse.*
	C57BL/6J-Drd1a^{tm1Jcd}/002959			
Drd3	B6,129S-Drd3^{tm1Dac}/002425	none	[3]	Hyperactivity and increased locomotor activity and rearing behavior. Mice heterozygous for the D3 receptor mutation show similar, albeit less pronounced, behavioral alterations. See *Anxiety-related behaviors.*
	C57BL/6J-Drd3^{tm1Dac}/002958			
Fos	B6,129X1-Fos^{tm1Pa}/002099	188, 2561, 2562, 3623, 4149	[39]	Pleiotropic effects including hyperactivity and diminished response to external stimuli. See *Learning and memory deficits* and *Seizures.*
	B6,129S-Fos^{tm1Pa}/002293			
	STOCK Fos^{tm1Pa}/003286			
Gabrb3	B6,129S-Gabrb3^{tm1Geh}/002711	5010	[30a]	Hyperactivity and hyperresponsiveness to human contact and other sensory stimuli, and circling. Lack of coordination. Difficulty in swimming, walking on grids, platforms, and rotarods. GABAA-mediated inhibition is nearly abolished in reticular nucleus, but unaffected in relay cells. Oscillatory synchrony during sleep is dramatically intensified. See *Aberrant parental behaviors* and *Seizures.*

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Table 4 (*continued*)

Gene[a]	IMR strain/stock number	TBASE ID	Reference	Knockout phenotype
Gm2a	B6,129S-Gm2a^{tm1Rlp}/003177	4516, 4517	[52]	Unlike Tay–Sachs mice, Gm2a knockouts display marked ganglioside storage in the cerebellum and defects in balance and coordination. See *Miscellaneous.*
Gria2	STOCK, Gria2^{tm1Rod}/002913	none	[38]	Increased mortality, reduced exploration, and impaired motor coordination. See *Learning and memory deficits.*
	C57BL/6J-Gria2^{tm1Rod}/003143			
Grm4	STOCK Grm4^{tm1Hpn}/003576	none	[70]	Normal motor activity, novelty-induced exploratory behaviors, and fine motor coordination. See *Learning and memory deficits.*
Hexb	B6,129S-Hexb^{tm1Rlp}/002914	2920, 2921	[80]	Progressive and profound neurologic disturbances and deterioration in motor and deterioration in motor function consistent with human Sandhoff disease. Extensive ganglioside storage in many areas of the CNS, including the cerebellum and spinal cord, both involved in motor function.
Kcna1	C3HeB/FeJ-Kcna1^{tm1Tem}/003532	none	[87]	Episodic ataxia/myokymia. See *Seizures.*
Kcne1	129/Sv-Kcne1^{tm1Sth}/003009	4966, 4967	[93]	Murine model for the Jervell and Lange–Nielsen syndrome. Classic shaker/waltzer behavior. See *Miscellaneous.*
L1cam	B6,129S-L1cam^{tm1Sor}/003120	none	[12]	Defects in the guidance of corticospinal axons across the pyramidal decussation (a major motor control pathway projecting from the cortex to the spinal cord.
Ntrk3	B6,129S-Ntrk3^{tm1Bbd}/002481	1155, 1157	[44]	Absence of 1a muscle afferent projections to spinal motor neurons. Fewer large myelinated axons in the dorsal root and posterior columns of the spinal cord. Abnormal movements and postures.
Pkcc	B6,129P-Pkcc^{tm1Std}/002466	1050, 1052, 1056, 1058, 4162, 4650	[2]	Motor discoordination. Intact eyeblink conditioning. See *Learning and memory deficits* and *Response to ethanol.*
Sod2	C57BL/6-Sod2^{tm1Leb}/002973	3958, 3959	[47]	Perinatal death. Progressive motor disturbances (weakness, rapid fatigue, and circling behavior). See *Miscellaneous.*
Seizures				
Akp2	B6,129-Akp2^{tm1Sor}/002317	2533, 2534	[54]	Lethal seizures due to defective metabolism of vitamin B6; animal model for human hypophosphatasia.
	129-Akp2^{tm1Sor}/002484			
	C57BL/6J-Akp2^{tm1Sor}/002741			
Bdnf	STOCK Bdnf^{tm1Jae}/002267	1149, 1151, 3199	[20a]	Suppressed epileptogenesis in heterozygous mutant mice. See *Motor/coordination/balance impairments.*
	B6,129S4-Bdnf^{tm1Jae}/002266			
Gad2	C57BL/6J-Gad2^{tm1Bae}/003654	none	[41]	Spontaneous seizures that result in increased mortality. Seizures can be precipitated by fear or mild stress.
Camk2a	C57BL/6-Camk2a^{tm1}/002362	2082, 2083,	[86]	Profound hyperexcitability, evident in epileptic seizures involving limbic structures including the hippocampus. See *Learning and memory deficits.*
Cstb	129/SvJ-Cstb^{tm1Rm}/003486	5014, 5015	[71]	Myoclonic seizures. Loss of cerebellar granule cells with features of apoptosis. See *Motor/coordination/balance impairments.*
Fos	B6,129X1-Fos^{tm1Pa}/002099	188, 2561, 2562, 3623, 4149	[97]	Impairment of structural and functional plasticities in the kindling model of epilepsy. See *Learning and memory deficits* and *Motor/coordination/balance impairments.*
	B6,129S-Fos^{tm1Pa}/002293			
	STOCK Fos^{tm1Pa}/003286			
Gabrb3	B6,129S-Gabrb3^{tm1Geh}/002711	5010	[30a]	Occasional epileptic seizures. See *Aberrant parental behaviors* and *Motor/coordination/balance impairments.*
Grik2	B6,129-Grik2^{tm1Sth}/003254	5433, 5434	[62]	Reduced susceptibility to kainate-induced seizures. See *Miscellaneous.*
Htr2c	C57BL/6-Htr2c^{tm1Jul}/002627	4112, 5482	[91]	Spontaneous death from seizures. Extreme susceptibility to audiogenic seizures starting at 2–3 months of age. See *Feeding disorders.*

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Table 4 (*continued*)

Gene[a]	IMR strain/stock number	TBASE ID	Reference	Knockout phenotype
Kcna1	C3HeB/FeJ-Kcna1^{tm1Tem}/003532	none	[87]	Frequent spontaneous seizures throughout adult life. Increased excitability in the CA3 recurrent axon in the CA3 recurrent axon collateral system, perhaps contributing to the limbic and tonic–clonic components of the observed epileptic phenotype. See*Motor/coordination/balance impairments.*
Miscellaneous				
Abcd1	B6,129-Abcd1^{tm1Kan}/003716	none	[21]	Absence of CNS impairment and neurological symptoms up to 6 months; development of VLCFA storage disease.
Adra2a	C57BL/6-Adra2a^{tm1Lel}/002777	none	[55]	Lack of hypotensive response to α2AR agonists.
Adra2c	STOCK Adra2c^{tm1Gsb}/002512	4465, 4466, 4467	[50]	None noted.
Apob	C57BL/6J-Apob^{tm1Unc}/002053	952, 954	[31]	Exencephalus and hydrocephalus and hypobetalipoproteinemia.
Atm	129S6/SvEvTac-Atm^{tm1Awb}/002753	3894, 3895, 5221	[6]	A paradigm of ataxia, telangiectasia (pleiotropic), growth retardation, neurologic dysfunction, male and female infertility defects in T lymphocyte maturation, and extreme sensitivity to gamma-irradiation. Malignant thymic lymphomas between 2 and 4 months of age.
Cdk5	B6,129-Cdk5^{tm1Kul}/003536	3966, 3967	[66]	Abnormal corticogenesis, neuronal pathology and perinatal death. Lack of cortical laminar structure and cerebellar foliation.
Col4a3	129-Col4a3^{tm1Dec}/002908	None	[13]	Progressive glomerulonephritis with microhematuria and proteinuria, consistent with the autosomal form of Alport syndrome.
Cp	STOCK Cp^{tm1Hrs}/003582	none	[28]	Mouse model for aceruloplasminemia: an inherited neurodegenerative disease with impairment of iron homeostasis.
Crh	B6,129S-Crh^{tm1Maj}/002783	2253, 2254	[61]	Impaired sexually dimorphic adrenal responsiveness to stress. Fetal glucocorticoid requirement for lung maturation, absence of adult glucocorticoid need.
Dab1	CBy,129S4-Dab1^{tm1Cpr}/003581	4607, 4608	[33]	Anomalies in the development of the cerebrum, hippocampus, and cerebellum. Development of the mdab1-1 cerebellum parallels development of reeler.
Drd2	C57BL/6-Drd2^{tm1Low}/003190	none	[42]	Chronic hyperprolactinemia and anterior lobe lactotroph hyperplasia without evidence of adenomatous transformation. Absence of hyperplasia of the intermediate lobe melanotrophs. Development of uterine adenomyosis in response to prolonged prolactin exposure in aged female knockouts.
Edn3	129-Edn3^{tm1Ywa}/002516	none	[7]	Aganglionic megacolon and pigmentary disorders. Putative model for the human Hirschprung's disease.
Ednrb	129/Sv-Ednrb^{tm1Ywa}/002517	none	[32]	Aganglionic megacolon associated with coat color spotting. Role for Ednrb in the development of two neural crest-derived cell lineages myenteric ganglion neurons and epidermal melanocytes. Putative model for a hereditary form of Hirschprung's disease.
	B6,129-Ednrb^{tm1Ywa}/003295			
Emx1	B6,129P-Emx1^{tm1Jlr}/003080	3950, 3951	[75]	Absence of obvious behavioral defects. Nearly complete absence of corpus callosum. Heterozygotes show partial penetrance for the corpus callosum abnormality.
En1	STOCK En1^{tm1Alj}/002656/003343	2761, 2762	[100]	Absence of most of the colliculi and cerebellum and the third and fourth cranial nerves in brains of neonatally lethal mutants. Deletion of mid-hindbrain tissue at E9.5. Disruption of patterning of the forelimb paws and sternum; truncation of the 13th ribs.
	C57BL/6J-En1^{tm1Alj}/003343			
En2	B6,129S-En2^{tm1Alj}/002657	1198, 1200	[60]	Altered adult cerebellar foliation pattern. General development delay and abnormal formation of specific fissures with the most the severe morphological disruptions in the posterior region of the cerebellum. Possible lobe transformations. Delay in fusion of the cerebellar rudiments at the midline by 15.5 dpc.

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Table 4 (*continued*)

Gene[a]	IMR strain/stock number	TBASE ID	Reference	Knockout phenotype
Evi1	STOCKEvi1^{tm1Mmor}/002906	none	[34]	Embryonic demise at 10.5 dpc. Disruption in the development of paraxial mesenchyme, defects in the heart, somites, and cranial ganglia. Failure of peripheral nervous system to develop.
Fmr1	FVB,129P-Fmr1^{tm1Cgr}/002700	2631, 2632, 4389, 4826	[92]	Model for Fragile X syndrome. Aberrant dendritic spine morphologic development. Impaired developmental organizational processes of synapse stabilization and elimination or pruning. Macroorchidism due to increased rate of Sertoli cell proliferation. See *Learning and memory deficits*.
	FVB/NJ-Fmr1^{tm1Cgr}/003024			
	C57BL/6J-Fmr1^{tm1Cgr}/003025			
Gfap	B6,129S-Gfap^{tm1Mes}/002642	3784, 3785	[58]	Normal behavior and CNS morphology. Enhanced LTP of both population spike amplitude and excitatory postsynaptic potential slope in the CA1 region of the hippocampus. Role in astrocyte–neuronal interactions.
Gm2a	B6,129S-Gm2a^{tm1Rlp}/003177	4516, 4517	[52]	Neuronal storage in restricted regions of the brain (piriform, entorhinal cortex, amygdala, and hypothalamic nuclei) reminiscent of the asymptomatic Tay–Sachs model mice. See *Motor/coordination/balance impairments*.
Grik2	B6,129-Grik2^{tm1Sfh}/003254	5433, 5434	[62]	Altered synaptic physiology. See *Seizures*.
Hdh	C57BL/6-Hdh^{tm1Mem}/002688	2917, 2918	[19]	Hdh inactivation is embryonic lethal and does not mimic the neuropathology of adult Huntington's disease.
Hexa	B6,129S-Hexa^{tm1Rlp}/002367	1440, 1442, 3773	[101]	Murine model of Tay–Sachs disease. Age-dependent accumulation of GM2 ganglioside in restricted regions of the brain, including the cerebral cortex and CA3 region of the hippocampus. Presence of neurons with membranous cytoplasmic bodies. Difference in the distribution of storage neurons suggesting a difference of ganglioside metabolism between humans and mice.
Hexb	B6,129S-Hexb^{tm1Rlp}/002914	2920, 2921	[80]	Progressive and profound neurologic disturbances and deterioration in motor and deterioration in motor function consistent with human Sandhoff disease. Extensive ganglioside storage in many areas of the CNS, including the cerebellum and spinal cord, both involved in motor function.
Hprt	B6,129-Hprt^{tm1Del}/003138	none	[67]	Progressive late onset neurological phenotype similar to human translated CAG repeat disorders. Neuronal intranuclear inclusions. Premature death.
Kcne1	129/Sv-Kcne1^{tm1Sfh}/003009	4966, 4967	[93]	Model for the Jervell and Lange–Nielsen syndrome. Mild cardiac cellular phenotype. Striatal marginal cells and the vestibular dark cells of the inner ear fail to generate an equivalent short circuit current in vitro, indicating a lack of transepithelial K^+ secretion. See *Motor/coordination balance impairments*.
Lifr	B6,129S-Lifr^{tm1Imx}/002402	none	[96]	Disrupted placentation. Severe osteopenia of perinatal bone. Reduced numbers of astrocytes in spinal cord and brain stem. Metabolic defects. Perinatal demise.
Mag	STOCK Mag^{tm1Rod}/002403	2677, 2678	[49]	Myelination and its compaction are normal. Aberrant organization of the periaxonal region. Subtle intention tremor.
Mash1	C57BL/6-Mash1^{tm1And}/002991	989, 991, 4331, 4332	[26]	Severe impairment of the olfactory epithelium and sympathetic, parasympathetic, and enteric ganglia.
Nf1	C57BL/6-Nf1^{tm1Fer}/002646	1164, 1166	[9]	Partial recapitulation of the human Von Reckinghausen neurofibromatosis or neurofibromatosis type 1 disease. Overgrowth of the paravertebral and the prevertebral sympathetic ganglia. See *Learning and memory deficits*.
Ngfb	C57BL/6-Ngfb^{tm1}/003312	2124, 2125, 3229	[16]	Perinatal loss of sensory and sympathetic neurons. Normal development of basal forebrain cholinergic neurons.
Ngfr	C,129S-Ngfr^{tm1Jae}/002124	930, 932, 933, 934, 1167, 4067	[48]	Deficits in the peripheral sensory nervous system. Altered response to NGF but not to other neurotrophins. Pineal glands lack innervation and sweat gland innervation is absent or reduced in particular footpads.
	C57BL/6J-Ngfr^{tm1Jae}/002213			

(*continued on next page*)

Table 4 (*continued*)

Gene[a]	IMR strain/stock number	TBASE ID	Reference	Knockout phenotype
Nos1	B6,129S-Nos1^{tm1Plh}/002633	986, 988, 2764, 2765, 2829, 3065, 3634	[35]	Grossly normal appearance, locomotor activity, breeding, LTP, and LTD. Resistance to neural stroke damage following middle cerebral artery ligation. Grossly enlarged stomachs, with hyper-trophy of the pyloric sphincter and the circular muscle layer. See *Aberrant social, reproductive, and parental behaviors* and *Aggression*.
	C57BL/6J-Nos1^{tm1Plh}/002986			
Ntf3	STOCK Ntf3^{tm1Jae}/002276	772, 774, 4980	[20b]	Defects in the peripheral nervous system and loss of limb proprioceptive afferents. Significant retardation in spontaneous hair follicle regression (catagen) in newborn heterozygotes.
	C57BL/6J-Ntf3^{tm1Jae}/002275			
Ntf5	129S4/SvJae-Ntf5^{tm1Jae}/002497	2523, 2524	[51]	Loss of sensory neurons in the nodose-petrosal and geniculate ganglia. Intact motor neurons of the facial nucleus and sym-pathetic neurons of the superior cervical ganglion.
Ntrk1	B6,129S-Ntrk1^{tm1Bbd}/002480	1158, 1160, 5031, 5032	[88]	Severe sensory and sympathetic neuropathies. Extensive neuronal cell loss in trigeminal, sympathetic, and dorsal root ganglia. Decrease in the cholinergic basal forebrain projections to the hippocampus and cortex. Drastic reduction in the number of nerve trunks and branches in the corneal stroma. Marked reduction of the blinking response to mechanical, thermal, and chemical noxious stimuli. Normal basic motor functions. Premature death.
Ntrk2	B6,129S-Ntrk2^{tm1Bbd}/002544	1023, 1025	[45]	Neuronal deficiencies in the central (facial motor nucleus and spinal cord) and peripheral (trigeminal and dorsal root ganglia) nervous systems. See *Feeding disorders*.
	C57BL/6J-Ntrk2^{tm1Bbd}/003098			
Plp	B6,129-Plp^{tm1Kan}/003255	none	[46]	Failure to exhibit dysmyelinated phenotype.
Psap	C57BL/6J-Psap^{tm1Suz}/002792	none	[22]	Progressive neurological symptoms and diminished survival. Extensive neurovisceral storage. Resemblance to human cases of total SAP deficiency.
Psen1	B6,129-Psen1^{tm1Shn}/003615	4398, 4399	[84]	Perinatal lethality. Skeletal malformations and impaired neurogenesis. Bilateral cerebral cavitation caused by massive neuronal loss in specific brain subregions following E16.5.
Snca	B6,129X-Snca^{tm1Rosl}/003692	none	[1]	Functional deficits in the nigrostriatal dopamine system. Reduction in total striatal dopamine and attenuated locomotor response when given amphetamine.
Sod2	C57BL/6-Sod2^{tm1Leb}/002973	3958, 3959	[47]	Perinatal death. Severe anemia, degeneration of neurons in the basal ganglia, and brainstem. Extensive mitochondrial injury within degenerating neurons and cardiac myocytes. See *Motor/coordination/balance impairments*.
Tcfap2a	BALB/c-Tcfap2a^{tm1Jae}/002794	4023, 4024	[81]	Perinatal death with cranioabdominoschisis and severe dysmorphogenesis of the face, skull, sensory organs, and cranial ganglia. Failure of cranial closure between 9 and 9.5 dpc.
Twist	C57BL/6J-Twist^{tm1Bhr}/002222	4116, 4133	[11]	Failure of the cranial neural folds to fuse. Defects in head mesenchyme, somites, and limb buds. Symptoms encountered in the human Saethre–Chotzen syndrome.

Abbreviations: CNS = central nervous system; dpc = days postcoitum; E16.5 = embryonic day 16.5; GABAA = gamma-aminobutyric acid (A); LTD = long-term depression; LTP = long-term potentiation; NGF = nerve growth factor; NMDA = *N*-methyl-D-aspartate; NMDAR = *N*-methyl-D-aspartate receptor; SAP = sphingolipid activator protein; TBASE ID: TBASE accession number; VLCFA = very long-chain fatty acids.

[a] Gene symbols are taken from the MGD database at TJL (http://www.informatics.jax.org/).

exhibiting a defect in spatial cognitive function and paradoxical responses to psychostimulants (http://tbase.jax.org/docs/DAT.html).

An information server (http://tbase.jax.org/docs/announce.html) provides links to TBASE-related announcements and general information. Announcements are categorized by subject to include information on (a) upcoming meetings, seminars, conferences, and workshops, (b) courses and demonstrations on transgenesis and gene targeting, (c) books, reviews, and laboratory manuals, (d) technical novelties, and

(e) video guides. General information categories include (a) transgenic facilities worldwide, (b) nomenclature guidelines, (c) animal welfare legislation and regulations, and (d) multiple links to additional sites of interest. Finally, a glossary serves the less sophisticated TBASE users, who may not be thoroughly familiar with transgenic or knockout terminology.

Instructions for searching TBASE are available at http://tbase.jax.org/docs/instr.html. Basic queries can be initiated by searching the TBASE CitDB and combining search terms by means of Boolean operators: users are sequentially directed from journal articles to TBASE accession numbers to individual TBASE records, each of which corresponds to a unique mutant mouse. Alternatively, users may perform more sophisticated queries by using the search form available at http://tbase.jax.org/docs/tb.html and combining or excluding terms of interest, as desired. Any questions concerning access or data submission to TBASE should be directed to Technical Support by sending an e-mail message to tbase@jax.org. Comments or suggestions may be addressed directly to Anna V. Anagnostopoulos at anna@jax.org. Direct paper submission forms are available on the Web via http://tbase.jax.org/docs/newforms.html and by mail or fax upon request.

2.3. Future directions

Since the relocation of TBASE, scientists and database developers at TJL have undertaken the challenging task of merging the IMR and TBASE databases into a novel, schematically reformed database, provisionally named Transgenic and Targeted Mutation Database (TTMD). Recently, curatorial efforts have largely focused on identifying coincident database contents and comparing schematic infrastructure at various levels. A preliminary list of behavioral and neurological mouse knockouts common to both resources is presented in Table 4. For the purpose of this publication, mutants in Table 4 are conveniently categorized by behavioral profiles that include: altered responses to drugs and ethanol; aberrant social, reproductive, and parental behaviors; learning and memory deficits; feeding disorders; aggression; anxiety-related behaviors; pain/analgesia; motor impairments; seizures; and, finally, a miscellaneous category that denotes other neurological aberrations.

A sophisticated, multitable schema is being designed to accommodate emerging technological advances and ensure adequate representation of transgenic and knockout data. Major data reorganization and incorporation of previously unavailable material, such as use of hierarchical controlled vocabularies and altered gene expression data, will be actively pursued. Our goal is to eventually incorporate all data on transgenics, knockouts, strains that serve as recipients for various transgenes, transpolygenics, as well as conditional and chemically induced mutants. Priority enhancements in TTMD also include the capture of established interspecific homology relationships, based upon criteria generated by MGD, GDB, OMIM, and other databases of model organisms, thus expanding its versatility and scope. Improved applications for data browsing and editing are currently under construction. The first TTMD database release is expected to be launched onto the World Wide Web within a year of this publication. Long-range goals are to include information on all TJL strains, including spontaneous mutation and inbred strains, in TTMD and on strains available at other institutions through the International Mouse Strain Resources, which is also available on TJL's web page and currently contains links to TJL strains and Medical Research Council Mammalian Genetics Unit strains at Harwell in the UK.

References

[1] Abeliovich A, Schmitz Y, Farinas I, Choi-Lundberg D, Ho WH, Castillo PE, Shinsky N, Verdugo JM, Armanini M, Ryan A, Hynes M, Phillips H, Sulzer D, Rosenthal A. Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron 2000;25:239–52.

[2] Abeliovich A, Chen C, Goda Y, Silva AJ, Stevens CF, Tonegawa S. Modified hippocampal long-term potentiation in PKC gamma-mutant mice. Cell 1993;75:1253–62.

[3] Accili D, Fishburn CS, Drago J, Steiner H, Lachowicz JE, Park BH, Gauda EB, Lee EJ, Cool MH, Sibley DR, Gerfen CR, Westphal H, Fuchs S. A targeted mutation of the D3 dopamine receptor gene is associated with hyperactivity in mice. Proc Natl Acad Sci USA 1996; 93:1945–9.

[4] Airaksinen MS, Eilers J, Garaschuk O, Thoenen H, Konnerth A, Meyer M. Ataxia and altered dendritic calcium signaling in mice carrying a targeted null mutation of the calbindin D28k gene. Proc Natl Acad Sci USA 1997;94:1488–93.

[5] Anagnostopoulos AV. TBASE: the relationalized database of transgenic animals and targeted mutations. In: Houdebine LM, editor. Transgenic animals: generation and use. Amsterdam: Harwood Academic Publishers, Overseas Publishers Association (OPA) Amsterdam, 1997. pp. 533–52.

[6] Barlow C, Hirotsune S, Paylor R, Liyanage M, Eckhaus M, Collins F, Shiloh Y, Crawley JN, Ried T, Tagle D, Wynshaw-Boris A. Atm-deficient mice: a paradigm of ataxia telangiectasia. Cell 1996;86: 159–71.

[7] Baynash AG, Hosoda K, Giaid A, Richardson JA, Emoto N, Hammer RE, Yanagisawa M. Interaction of endothelin-3 with endothelin-B receptor is essential for development of epidermal melanocytes and enteric neurons. Cell 1994;79:1277–85.

[8] Bolivar V, Cook M, Flaherty L. List of transgenic and knockout mice: behavioral profiles. Mamm Genome 2000;11:260–74.

[9] Brannan CI, Perkins AS, Vogel KS, Ratner N, Nordlund ML, Reid SW, Buchberg AM, Jenkins NA, Parada LF, Copeland NG. Targeted disruption of the neurofibromatosis type-1 gene leads to developmental abnormalities in heart and various neural crest-derived tissues. Genes Dev 1994;8:1019–29.

[10] Brown JR, Ye H, Bronson RT, Dikkes P, Greenberg ME. A defect in nurturing in mice lacking the immediate early gene fosB. Cell 1996; 86:297–309.

[11] Chen ZF, Behringer RR. Twist is required in head mesenchyme for cranial neural tube morphogenesis. Genes Dev 1995;9:686–99.

[12] Cohen NR, Taylor JS, Scott LB, Guillery RW, Soriano P, Furley AJ. Errors in corticospinal axon guidance in mice lacking the neural cell adhesion molecule L1. Curr Biol 1998;8:26–33.

[13] Cosgrove D, Meehan DT, Grunkemeyer JA, Kornak JM, Sayers WJ, Hunter WJ, Samuelson GC. Collagen COL4A3 knockout: a mouse model for autosomal Alport syndrome. Genes Dev 1996; 10:2981–92.

[14] Crawley JN. Behavioral phenotyping of transgenic and knockout

mice: experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. Brain Res 1999;835:18–26 (review).

[15] Cremer H, Lange R, Christoph A, Plomann M, Vopper G, Roes J, Brown R, Baldwin S, Kraemer P, Scheff S, et al. Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. Nature 1994;367:455–9.

[16] Crowley C, Spencer SD, Nishimura MC, Chen KS, Pitts-Meek S, Armanini MP, Ling LH, MacMahon SB, Shelton DL, Levinson AD, et al. Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell 1994;76:1001–11.

[17] Crusio WE, Gerlai R, editors. Handbook of molecular–genetic techniques for brain and behavior research. Amsterdam: Elsevier, 1999.

[18] Drago J, Gerfen CR, Lachowicz JE, Steiner H, Hollon TR, Love PE, Ooi GT, Grinberg A, Lee EJ, Huang SP, et al. Altered striatal function in a mutant mouse lacking D1A dopamine receptors. Proc Natl Acad Sci USA 1994;91:12564–8.

[19] Duyao MP, Auerbach AB, Ryan A, Persichetti F, Barnes GT, McNeil SM, Ge P, Vonsattel JP, Gusella JF, Joyner AL, et al. Inactivation of the mouse Huntington's disease gene homolog Hdh. Science 1995; 269:407–10.

[20a] Emfors P, Lee KF, Jaenisch R. Mice lacking brain-derived neurotrophic factor develop with sensory deficits. Nature 1994;368:147–50.

[20b] Emfors P, Lee KF, Kucera J, Jaenisch R. Lack of neurotrophin-3 leads to deficiencies in the peripheral nervous system and loss of limb proprioceptive afferents. Cell 1994;77:503–12.

[21] Forss-Petter S, Werner H, Berger J, Lassmann H, Molzer B, Schwab H, Bernheimer H, Zimmermann F, Nave KA. Targeted inactivation of the X-linked adrenoleukodystrophy gene in mice. J Neurosci Res 1997;50:829–43.

[22] Fujita N, Suzuki K, Vanier MT, Popko B, Maeda N, Klein A, Henseler M, Sandhoff K, Nakayasu H, Suzuki K. Targeted disruption of the mouse sphingolipid activator protein gene: a complex phenotype, including severe leukodystrophy and wide-spread storage of multiple sphingolipids. Hum Mol Genet 1996;5:711–25.

[23a] Geppert M, Bolshakov VY, Siegelbaum SA, Takei K, DeCamilli P, Hammer RE, Sudhof TC. The role of Rab3A in neurotransmitter release. Nature 1994;369:493–7.

[23b] Geppert M, Goda Y, Hammer RE, Li C, Rosahl TW, Stevens CF, Sudhof TC. Synaptotagmin I: a major Ca^{2+} sensor for transmitter release at a central synapse. Cell 1994;79:717–27.

[24] Gerlai R, Roder JC, Hampson DR. Altered spatial learning and memory in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. Behav Neurosci 1998;112:525–32.

[25] Grant SG, O'Dell TJ, Karl KA, Stein PL, Soriano P, Kandel ER. Impaired long-term potentiation, spatial learning, and hippocampal development in fyn mutant mice. Science 1992;258:1903–10.

[26] Guillemot F, Lo LC, Johnson JE, Auerbach A, Anderson DJ, Joyner AL. Mammalian achaete-scute homolog 1 is required for the early development of olfactory and autonomic neurons. Cell 1993;75: 463–76.

[27] Hampton LL, Ladenheim EE, Akeson M, Way JM, Weber HC, Sutliff VE, Jensen RT, Wine LJ, Arnheiter H, Battey JF. Loss of bombesin-induced feeding suppression in gastrin-releasing peptide receptor-deficient mice. Proc Natl Acad Sci USA 1998;95:3188–92.

[28] Harris ZL, Durley AP, Man TK, Gitlin JD. Targeted gene disruption reveals an essential role for ceruloplasmin in cellular iron efflux. Proc Natl Acad Sci USA 1999;96:10812–7.

[29] Homanics GE, Harrison NL, Quinlan JJ, Krasowski MD, Rick CE, de Blas AL, Mehta AK, Kist F, Mihalek RM, Aul JJ, Firestone LL. Normal electrophysiological and behavioral responses to ethanol in mice lacking the long splice variant of the gamma2 subunit of the gamma-aminobutyrate type A receptor. Neuropharmacology 1999;38: 253–65.

[30a] Homanics GE, DeLorey TM, Firestone LL, Quinlan JJ, Handforth A, Harrison MD, Krasowski CE, Rick ER, Korpi R, Makela MH, Brilliant N, Hagiwara C, Ferguson K, Snyder RW, Olsen RW. Mice devoid of gamma-aminobutyrate type A receptor beta3 subunit have epilepsy, cleft palate, and hypersensitive behavior. Proc Natl Acad Sci USA 1997;94:4143–8.

[30b] Homanics GE, Ferguson C, Quinlan JJ, Daggett J, Snyder K, Lagenaur C, Mi ZP, Wang XH, Grayson DR, Firestone LL. Gene knockout of the alpha6 subunit of the gamma-aminobutyric acid type A receptor: lack of effect on responses to ethanol, pentobarbital, and general anesthetics. Mol Pharmacol 1997;51:588–96.

[31] Homanics GE, Smith TJ, Zhang SH, Lee D, Young SG, Maeda N. Targeted modification of the apolipoprotein B gene results in hypobetalipoproteinemia and developmental abnormalities in mice. Proc Natl Acad Sci USA 1993;90:2389–93.

[32] Hosoda K, Hammer RE, Richardson JA, Baynash AG, Cheung JC, Giaid A, Yanagisawa M. Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. Cell 1994;79:1267–76.

[33] Howell BW, Hawkes R, Soriano P, Cooper JA. Neuronal position in the developing brain is regulated by mouse disabled-1. Nature 1997; 389:733–7.

[34] Hoyt PR, Bartholomew C, Davis AJ, Yutzey K, Gamer LW, Potter SS, Ihle JN, Mucenski ML. The Evi1 proto-oncogene is required at midgestation for neural, heart, and paraxial mesenchyme development. Mech Dev 1997;65:55–70.

[35] Huang PL, Dawson TM, Bredt DS, Snyder SH, Fishman MC. Targeted disruption of the neuronal nitric oxide synthase gene. Cell 1993; 75:1273–86.

[36] Ingram DK, Jucker M. Developing mouse models of aging: a consideration of strain differences in age-related behavioral and neural parameters (review)Neurobiol Aging 1999;20:137–45.

[37] Jacobson D, Anagnostopoulos AV. Internet resources for transgenic or targeted mutation research. Trends Genet 1996;12:117–8.

[38] Jia Z, Agopyan N, Miu P, Xiong Z, Henderson J, Gerlai R, Taverna FA, Velumian A, MacDonald J, Carlen P, Abramow-Newerly W, Roder J. Enhanced LTP in mice deficient in the AMPA receptor GluR2. Neuron 1996;17:945–56.

[39] Johnson RS, Spiegelman BM, Papaioannou V. Pleiotropic effects of a null mutation in the c-fos proto-oncogene. Cell 1992;71:577–86.

[40] Kaartinen V, Mononen I, Voncken JW, Noronkoski T, Heisterkamp I, Gonzalez-Gomez N, Groffen J. A mouse model for the human lysosomal disease aspartylglycosaminuria. Nat Med 1996;2:1375–8.

[41] Kash SF, Johnson RS, Tecott LH, Noebels JL, Mayfield RD, Hanahan D, Baekkeskov S. Epilepsy in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. Proc Natl Acad Sci USA 1997;94: 14060–5.

[42] Kelly MA, Rubinstein M, Asa SL, Zhang G, Saez C, Bunzow JR, Allen RG, Hnasko R, Ben-Jonathan N, Grandy DK, Low MJ. Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. Neuron 1997;19:103–13.

[43] Keverne EB. An evaluation of what the mouse knockout experiments are telling us about mammalian behaviour (review)BioEssays 1997; 19:1091–8.

[44] Klein R, Silos-Santiago I, Smeyne RJ, Lira SA, Brambilla R, Bryant S, Zhang L, Snider WD, Barbacid M. Disruption of the neurotrophin-3 receptor gene trkC eliminates la muscle afferents and results in abnormal movements. Nature 1994;368:249–51.

[45] Klein R, Smeyne RJ, Wurst W, Long LK, Auerbach BA, Joyner AL, Barbacid M. Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death. Cell 1993; 75: 113–22.

[46] Klugmann M, Schwab MH, Puhlhofer A, Schneider A, Zimmermann F, Griffiths IR, Nave KA. Assembly of CNS myelin in the absence of proteolipid protein. Neuron 1997;18:59–70.

[47] Lebovitz RM, Zhang H, Vogel H, Cartwright J, Dionne L, Lu N, Huang S, Matzuk MM. Neurogegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. Proc Natl Acad Sci USA 1996;93:9782–7.

[48] Lee KF, Li E, Huber LJ, Landis SC, Sharpe AH, Chao MV, Jaenisch R. Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. Cell 1992;69:737–49.
[49] Li C, Tropak MB, Gerlai R, Clapoff S, Abramow-Newerly W, Trapp B, Peterson A, Roder J. Myelination in the absence of myelin-associated glycoprotein. Nature 1994;369:747–50.
[50] Link RE, Stevens MS, Kulatunga M, Scheinin M, Barsh GS, Kobilka BK. Targeted inactivation of the gene encoding the mouse alpha 2c-adrenoceptor homolog. Mol Pharmacol 1995;48:48–55.
[51] Liu X, Ernfors P, Wu H, Jaenisch R. Sensory but not motor neuron deficits in mice lacking NT4 and BDNF. Nature 1995;375:238–41.
[52] Liu Y, Hoffmann A, Grinberg A, Westphal H, McDonald MP, Miller KM, Crawley JN, Sandhoff K, Suzuki K, Proia RL. Mouse model of GM2 activator deficiency manifests cerebellar pathology and motor impairment. Proc Natl Acad Sci USA 1997;94:8138–43.
[53] Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM, Roder JC. Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. J Neurosci 1997;17:5196–205.
[54] MacGregor GR, Zambrowicz BP, Soriano P. Tissue non-specific alkaline phosphatase is expressed in both embryonic and extraembryonic lineages during mouse embryogenesis but is not required for migration of primordial germ cells. Development 1995;121:1487–96.
[55] MacMillan LB, Hein L, Smith MS, Piascik MT, Limbird LE. Central hypotensive effects of the alpha2-adrenergic receptor subtype. Science 1996;273:801–3.
[56] Matzuk MM, Dionne L, Guo Q, Kumar TR, Lebovitz RM. Ovarian function in superoxide dismutase 1 and 2 knockout mice. Endocrinology 1998;139:4008–11.
[57] Matzuk MM, Kumar TR, Bradley A. Different phenotypes for mice deficient in either activins or activin receptor type II. Nature 1995; 374:356–60.
[58] McCall MA, Gregg RG, Behringer RR, Brenner M, Delaney CL, Galbreath EJ, Zhang CL, Pearce RA, Chiu SY, Messing A. Targeted deletion in astrocyte intermediate filament (Gfap) alters neuronal physiology. Proc Natl Acad Sci USA 1996;93:6361–6.
[59] Mihalek RM, Banerjee PK, Korpi ER, Quinlan JJ, Firestone LL, Mi ZP, Lagenaur C, Tretter V, Sieghart W, Anagnostaras SG, Sage JR, Fanselow MS, Guidotti A, Spigelman I, Li Z, DeLorey TM, Olsen GE, Homanics GE. Attenuated sensitivity to neuroactive steroids in gamma-aminobutyrate type A receptor delta subunit knockout mice. Proc Natl Acad Sci USA 1999;96:12905–10.
[60] Millen KJ, Wurst W, Herrup K, Joyner AL. Abnormal embryonic cerebellar development and patterning of postnatal foliation in two mouse Engrailed-2 mutants. Development 1994;120:695–706.
[61] Muglia L, Jacobson L, Dikkes P, Majzoub JA. Corticotropin-releasing hormone deficiency reveals major fetal but not adult glucocorticoid need. Nature 1995;373:427–32.
[62] Mulle C, Sailer A, Perez-Otano I, Dickinson-Anson H, Castillo PE, Bureau I, Maron C, Gage FH, Mann JR, Bettler B. Altered synaptic physiology and reduced susceptibility to kainate-induced seizures in GluR6-deficient mice. Nature 1998;392:601–5.
[63] Delivering the knockout punch (editorial)Nat Genet 1996;13:251–2.
[64] Nelson RJ, Young KA. Behavior in mice with targeted disruption of single genes (review)Neurosci Biobehav Rev 1998;22:453–62.
[65] Nelson RJ. The use of genetic "knockout" mice in behavioral endocrinology research (review)Horm Behav 1997;31:188–96.
[66] Ohshima T, Ward JM, Huh CG, Longenecker G. Targeted disruption of the cyclin-dependent kinase 5 gene results in abnormal corticogenesis, neuronal pathology and perinatal death. Proc Natl Acad Sci USA 1996;93:11173–8.
[67] Ordway JM, Tallaksen-Greene S, Gutekunst CA, Bernstein EM, Cearley JA, Wiener HW, Dure LS, Lindsey R, Hersch SM, Jope RS, Albin RL, Detloff PJ. Ectopically expressed CAG repeats cause intranuclear inclusions and a progressive late onset neurological phenotype in the mouse. Cell 1997;91:753–63.
[68] Orr-Urtreger A, Goldner FM, Saeki M, Lorenzo I, Goldberg L, De Biasi M, Dani JA, Patrick JW, Beaudet AL. Mice deficient in the alpha7 neuronal nicotinic acetylcholine receptor lack alpha-bungarotoxin binding sites and hippocampal fast nicotinic currents. J Neurosci 1997;17:9165–71.
[69] Paylor R, Johnson RS, Papaioannou V, Spiegelman BM, Wehner JM. Behavioral assessment of c-fos mutant mice. Brain Res 1994;651: 275–82.
[70] Pekhletski R, Gerlai R, Overstreet LS, Huang XP, Agopyan N, Slater NT, Abramow-Newerly W, Roder JC, Hampson DR. Impaired cerebellar synaptic plasticity and motor performance in mice lacking the mGluR4 subtype of metabotropic glutamate receptor. J Neurosci 1996;16:6364–73.
[71] Pennacchio LA, Bouley DM, Higgins KM, Scott MP, Noebels RM, Myers RM. Progressive ataxia, myoclonic epilepsy and cerebellar apoptosis in cystatin B-deficient mice. Nat Genet 1998;20: 251–8.
[72] Picciotto MR, Wickman K. Using knockout and transgenic mice to study neurophysiology and behavior (review)Physiol Rev 1998;78: 1131–63.
[73] Pich EM, Epping-Jordan MP. Transgenic mice in drug dependence research (review)Ann Med 1998;30:390–6.
[74] Piedrahita JA, Zhang SH, Hagaman JR, Oliver PM, Maeda N. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc Natl Acad Sci USA 1992;89:4471–5.
[75] Qiu M, Anderson S, Chen S, Meneses JJ, Hevner R, Kuwana E, Pedersen RA, Rubenstein JL. Mutation of the Emx-1 homeobox gene disrupts the corpus callosum. Dev Biol 1996;178:174–8.
[76] Reimold AM, Grusby MJ, Kosaras B, Fries JW, Mori R, Maniwa S, Clauss IM, Collins T, Sidman RL, Glimche MJ, Glimcher LH. Chondrodysplasia and neurological abnormalities in ATF-2-deficient mice. Nature 1996;379:262–5.
[77] Rosahl TW, Spillane D, Missler M, Herz J, Selig DK, Wolff JR, Hammer RE, Malenka RC, Sudhof TC. Essential functions of synapsins I and II in synaptic vesicle regulation. Nature 1995;375: 488–93.
[78] Rosahl TW, Geppert M, Spillane D, Herz J, Hammer RE, Malenka RC, Sudhof TC. Short-term synaptic plasticity is altered in mice lacking synapsin I. Cell 1993;75:661–70.
[79] Rubinstein M, Mogil JS, Japon M, Cheng Chan E, Allen RG, Low MJ. Absence of opioid stress-induced analgesia in mice lacking beta-endorphin by site-directed mutagenesis. Proc Natl Acad Sci USA 1996;93:3995–4000.
[80] Sango K, Yamanaka S, Hoffmann A, Okuda Y, Grinberg A, Westphal H, McDonald MP, Crawley JN, Sandhoff K, Suzuki K, et al. Mouse models of Tay–Sachs and Sandhoff diseases differ in neurologic phenotype and ganglioside metabolism. Nat Genet 1995;11:170–6.
[81] Schorle H, Meier P, Buchert M, Jaenisch R, Mitchell PJ. Transcription factor AP-2 essential for cranial closure and craniofacial development. Nature 1996;381:235–8.
[82] Sharp JJ, Mobraaten LE. To save or not to save: the role of repositories in a period of rapidly expanding development of genetically engineered strains of mice. In: Houdebine LM, editor. Transgenic animals: generation and use. Amsterdam: Harwood Academic Publishers, Overseas Publishers Association (OPA) Amsterdam, 1997. pp. 525–32.
[83] Sharp JJ, Davisson MT. The Jackson Laboratory Induced Mutant Resource. Lab Anim 1994;23:32–40.
[84] Shen J, Bronson RT, Chen DF, Xia W, Selkoe DJ, Tonegawa S. Skeletal and CNS defects in Presenilin-1-deficient mice. Cell 1997; 89:629–39.
[85] Silva AJ, Frankland PW, Marowitz Z, Friedman E, Lazlo G, Cioffi D, Jacks T, Bourtchuladze R. A mouse model for the learning and memory deficits associated with neurofibromatosis type I. Nat Genet 1997;15:281–4.
[86] Silva AJ, Stevens CF, Tonegawa S, Wang Y. Deficient hippocampal

long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. Science 1992;257:201–6.

[87] Smart SL, Lopantsev V, Zhang CL, Robbins CA, Wang H, Chiu SY, Schwartzkroin PA, Messing A, Tempel BL. Deletion of the K(V)1.1 potassium channel causes epilepsy in mice. Neuron 1998;20:809–19.

[88] Smeyne RJ, Klein R, Schnapp A, Long LK, Bryant S, Lewin A, Lira SA, Lewin A, Barbacid M. Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. Nature 1994;368:246–9.

[89] Supp DM, Witte DP, Branford WW, Smith EP, Potter SS. Sp4, a member of the Sp1-family of zinc finger transcription factors, is required for normal murine growth, viability, and male fertility. Dev Biol 1996;176:284–99.

[90] Takahashi JS, Pinto LH, Vitaterna MH. Forward and reverse genetic approaches to behavior in the mouse. Science 1994;264:1724–33.

[91] Tecott LH, Sun LM, Akana SF, Strack AM, Lowenstein DH, Dallman MF, Julius D. Eating disorder and epilepsy in mice lacking 5-HT[2C] serotonin receptors. Nature 1995;374:542–6.

[92] The Dutch-Belgian Fragile X Consortium. Fmr1 knockout mice: a model to study fragile X mental retardation. Cell 1994;78:23–33.

[93] Vetter DE, Mann JR, Wangemann P, Liu J, McLaughlin KJ, Lesage F, Marcus DC, Lazdunski M, Heinemann SF, Barhanin J. Inner ear defects induced by null mutation of the isk gene. Neuron 1996;17:1251–64.

[94] Veinbergs I, Mante M, Jung MW, Van Uden E, Masliah E. Synaptotagmin and synaptic transmission alterations in apolipoprotein E-deficient mice. Prog Neuropsychopharmacol Biol Psychiatry 1999;23:519–31.

[95] Veinbergs I, Jung MW, Young SJ, Van Uden E, Groves PM, Masliah E. Altered long-term potentiation in the hippocampus of apolipoprotein E-deficient mice. Neurosci Lett 1998;249:71–4.

[96] Ware CB, Horowitz MC, Renshaw BR, Hunt JS, Liggitt D, Koblar SA, Liggitt D, Gliniak BC, McKenna HJ, Papayannopoulou T, Thoma B, et al. Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. Development 1995;121:1283–99.

[97] Watanabe Y, Johnson RS, Butler LS, Binder DK, Spiegelman BM, Papaioannou VE, McNamara JO. Null mutation of c-fos impairs structural and functional plasticities in the kindling model of epilepsy. J Neurosci 1996;16:3827–36.

[98] Williams RS, Wagner PD. Transgenic animals in integrative biology: approaches and interpretations of outcome (review)J Appl Physiol 2000;88:1119–26.

[99] Woychik RP, Wassom JS, Jacobson DA, Kingsbury DT. TBASE, a computerized database for transgenic animals and targeted mutants. Nature 1993;363:375–6.

[100] Wurst W, Auerbach AB, Joyner AL. Multiple developmental defects in Engrailed-1 mutant mice: an early mid-hindbrain deletion and patterning defects in forelimbs and sternum. Development 1994;120:2065–75.

[101] Yamanaka S, Johnson MD, Grinberg A, Westphal H, Crawley JN, Taniike M, Suzuki K, Proia RL. Targeted disruption of the Hexa gene results in mice with biochemical and pathologic features of Tay–Sachs disease. Proc Natl Acad Sci USA 1994;91:9975–9.